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Program in the History of the Biological Sciences and Biotechnology

Mary C. Betlach

EARLY CLONING AND RECOMBINANT DNA TECHNOLOGY AT HERBERT W. BOYER'S UCSF LABORATORY IN THE 1970s

Interview Conducted by
Sally Smith Hughes, Ph.D.
in 1994

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Mary C. Betlach, 1972.

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Discussion of laboratory facilities, personnel, competition, and working atmosphere at Herbert Boyer's laboratory in the early 1970s; early work to purify restriction enzymes and plasmids and clone DNA; biosafety concerns; plasmid vector development, approval and certification, and conflicts surrounding dissemination to colleagues; perspectives on division between science and industry; opinions on scientists who merit the Nobel Prize; comments on Herbert Boyer, Stanley N. Cohen, Robert Helling, Ernest Jawetz, Art Riggs, William J. Rutter, and others.

Interviewed in 1994 by Sally Smith Hughes for the Program in the History of Biosciences and Biotechnology, Regional Oral History Office, The Bancroft Library, University of California, Berkeley.

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BIOTECHNOLOGY SERIES HISTORY--Sally Smith Hughes, Ph.D.

Genesis of the Program in the History of the Biological Sciences and Biotechnology

In 1996 The Bancroft Library launched the Program in the History of the Biological Sciences and Biotechnology. Bancroft has strong holdings in the history of the physical sciences--the papers of E.O. Lawrence, Luis Alvarez, Edwin McMillan, and other campus figures in physics and chemistry, as well as a number of related oral histories. Yet, although the university is located next to the greatest concentration of biotechnology companies in the world, Bancroft had no coordinated program to document the industry or its origins in academic biology.

When Charles Faulhaber arrived in 1995 as Bancroft's director, he agreed on the need to establish a Bancroft program to capture and preserve the collective memory and papers of university and corporate scientists and the pioneers who created the biotechnology industry. Documenting and preserving the history of a science and industry which influences virtually every field of the life sciences, generates constant public interest and controversy, and raises serious questions of public policy is vital for a proper understanding of science and business in the late twentieth and early twenty-first centuries.

The Bancroft Library is the ideal location to carry out this historical endeavor. It offers the combination of experienced oral history and archival personnel, and technical resources to execute a coordinated oral history and archival program. It has an established oral history series in the biological sciences, an archival division called the History of Science and Technology Program, and the expertise to develop comprehensive records management plans to safeguard the archives of individuals and businesses making significant contributions to molecular biology and biotechnology. It also has longstanding cooperative arrangements with UC San Francisco and Stanford University, the other research universities in the San Francisco Bay Area.

In April 1996, Daniel E. Koshland, Jr. provided seed money for a center at The Bancroft Library for historical research on the biological sciences and biotechnology. And then, in early 2001, the Program in the History of the Biological Sciences and Biotechnology was given great impetus by Genentech's generous pledge of one million dollars to support documentation of the biotechnology industry.

Thanks to these generous gifts, Bancroft has been building an integrated collection of research materials--primarily oral history transcripts, personal papers, and archival collections--related to the history of the biological sciences and biotechnology in university and industry settings. A board composed of distinguished figures in academia and industry advise on the direction of the oral history and archival components. The Program's initial concentration is on the San Francisco Bay Area and northern California. But its ultimate aim is to document the growth of molecular biology as an independent field of the life sciences, and the subsequent revolution which established biotechnology as a key contribution of American science and industry. The UCSF Library, with its strong holdings in the biomedical sciences, is a collaborator on the archival portion of the Program. David Farrell, Bancroft's curator of the History of Science and Technology, serves as liaison.

Oral History Process

The oral history methodology used in this program is that of the Regional Oral History Office, founded in 1954 and producer of over 1,800 oral histories. The method consists of research in primary and secondary sources; systematic recorded interviews; transcription, light editing by the interviewer, and review and approval by the interviewee; library deposition of bound volumes of transcripts with table of contents, introduction, interview history, and index; cataloging in UC Berkeley and national

online library networks; and publicity through ROHO news releases and announcements in scientific, medical, and historical journals and newsletters and via the ROHO and UCSF Library Web pages.

Oral history as a historical technique has been faulted for its reliance on the vagaries of memory, its distance from the events discussed, and its subjectivity. All three criticisms are valid; hence the necessity for using oral history documents in conjunction with other sources in order to reach a reasonable historical interpretation.[1] Yet these acknowledged weaknesses of oral history, particularly its subjectivity, are also its strength. Often individual perspectives provide information unobtainable through more traditional sources. Oral history in skillful hands provides the context in which events occur--the social, political, economic, and institutional forces which shape the course of events. It also places a personal face on history which not only enlivens past events but also helps to explain how individuals affect historical developments.

Emerging Themes

Although the oral history program is still in its initial phase, several themes are emerging. One is "technology transfer," the complicated process by which scientific discovery moves from the university laboratory to industry where it contributes to the manufacture of commercial products. The oral histories show that this trajectory is seldom a linear process, but rather is influenced by institutional and personal relationships, financial and political climate, and so on.

Another theme is the importance of personality in the conduct of science and industry. These oral histories testify to the fact that who you are, what you have and have not achieved, whom you know, and how you relate have repercussions for the success or failure of an enterprise, whether scientific or commercial. Oral history is probably better than any other methodology for documenting these personal dimensions of history. Its vivid descriptions of personalities and events not only make history vital and engaging, but also contribute to an understanding of why circumstances occurred in the manner they did.

Molecular biology and biotechnology are fields with high scientific and commercial stakes. As one might expect, the oral histories reveal the complex interweaving of scientific, business, social, and personal factors shaping these fields. The expectation is that the oral histories will serve as fertile ground for research by present and future scholars interested in any number of different aspects of this rich and fascinating history.

Location of the Oral Histories

Copies of the oral histories are available at the Bancroft, UCSF, and UCLA libraries. They also may be purchased at cost through the Regional Oral History Office. Some of the oral histories, with more to come, are available on The Bancroft Library's History of the Biological Sciences and Biotechnology Website: http://bancroft.berkeley.edu/Biotech/.

Sally Smith Hughes, Ph.D.
Historian of Science

Regional Oral History Office
The Bancroft Library
University of California, Berkeley
August 2002

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[1]The three criticisms leveled at oral history also apply in many cases to other types of documentary sources.

INTERVIEW HISTORY--Mary C. Betlach

Dr. Betlach was interviewed in 1994 about her position as key technician in Herbert W. Boyer's laboratory at UCSF at the time of the creation and early expansion of recombinant DNA technology. Although the inspiration for the interview came from an oral history conducted that year with Dr. Boyer, her central role in the development of several procedures that made recombinant DNA widely practicable are historically as well as technically important in their own right.

Betlach came to Boyer's lab in 1972, eager to work on restriction enzyme modification, the lab's central focus. It was to become the reason for Boyer's collaboration with Stanley N. Cohen of Stanford in the genesis in 1973-1974 of a straightforward method for combining and amplifying DNA. Betlach describes her participation in the development of recombinant DNA technology and the laboratory's role in disseminating it to molecular biology laboratories worldwide.

Although Cohen's technician at the time, Annie Chang, is co-author of three papers on recombinant DNA published in these years, Betlach, who also played a seminal technical role, is not an author. When the topic came up in the interview, she was characteristically nonchalant. Because as a general rule, technicians are not named as authors of scientific publications, Annie Chang's position on the papers could be regarded as the exception to the rule and Betlach's "omission" the more common situation. Whatever the reason, one hopes that the oral history establishes for the historical record that Betlach was far from a pair of hands in the Boyer laboratory; she created and modified procedures instrumental for the development and expansion of recombinant DNA technology. As she describes in the interview, it was Betlach who created some of the earliest plasmids critical to the application of recombinant DNA and sent them out to investigators around the world. She also reflects on the atmosphere of the laboratory which, like the man at its head, was simultaneously competitive, laid-back, and amazingly productive. Both Betlach and Chang went on to earn doctorates in the biomolecular sciences.

Betlach's view of the accomplishments and culture of the Boyer lab at the height of its preeminence has obvious historical merit. The interview is also a welcome extension of the Boyer oral history and the oral histories in progress with Stanley Cohen and Herbert Heyneker, a postdoctoral fellow in the Boyer laboratory in 1975-1977. Together they provide novel historical documentation of the earliest manifestation of a technology destined to transform biomedical science and to become a major basis for the biotechnology industry.

Sally Smith Hughes, Ph.D.
Historian of Science

Regional Oral History Office
The Bancroft Library
University of California, Berkeley
July 2002

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Berkeley, California 94720

BIOGRAPHICAL INFORMATION

(Please write clearly. Use black ink.)

Your full name MARY CAROLYN Betlach

Date of birth 6/12/45 Birthplace Madison, Wisconsin

Father's full name William Thompson Stafford

Occupation M.D. (Surgeon) Birthplace Chippewa Falls, WI

Mother's full name Carolyn Jesse Gillette McCormick

Occupation Nurse/housewife Birthplace Belleville, WI

Your spouse/partner I am divorced

Occupation ____ Birthplace ____

Your children John F Betlach born 9/2/65
Melanie Carolyn Betlach born 9/16/74

Where did you grow up? Madison, Wisconsin

Present community San Francisco, CA

Education University of Wisconsin 1963-1967
University of California, San Francisco Ph.D. Biochemistry

Occupation(s) Scientist; current position is Director, Grant Research Collaborations at Sunesis Pharmaceuticals in South San Francisco

Areas of expertise
Regulation of gene expression. Heterologous protein expression. Genetic engineering of biosynthetic pathways.

Other interests or activities hiking, birdwatching, gardening

Organizations in which you are active Golden Gate Audubon Society

SIGNATURE Mary C Betlach DATE: 1/15/2002

INTERVIEW WITH MARY BETLACH

The Herbert W. Boyer Laboratory at UCSF in the Early 1970s

[Date of Interview: March 25, 1994]##[1]

Old Facilities in the Microbiology Department

Betlach: We were in an old part of the Medical Sciences building and the labs were pretty funky--old-fashioned histology labs. We didn't have a lot of space. The chairman of the microbiology department, Ernest Jawetz, came to me on more than one occasion and asked for my help in cleaning the place up. Once he--and he made me laugh--appealed to me "as a wife and a mother," couldn't I please do something about this place? And the only reason it was really messy was because we were crowded. When I came it was disorganized and there just wasn't a lot of space. And a couple of the graduate students that Herb had, Joel Hedgpeth in particular, were kind of messy.

So I reorganized the whole place a little bit. As we got more people, of course it just got worse, and what could I say or do? A small, ill-designed space, you can only organize so well. There's just not too much more you can do. We were having to walk across to another building to take our gel pictures. What is here at Parnassus Pharmaceuticals is a paradise compared to what we had--three tiny rooms.

Hughes: Was that typical lab space at UCSF at that time? Or do you think that your lab was particularly deprived?

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[1]## This symbol indicates that a tape or tape segment has begun or ended. A guide to the tapes follows the transcript.

Betlach: I don't know. Herb probably knows a lot more about how it was set up.

We were in an old part of the building and there were new labs in the new towers, Health Sciences East and West, which had just been built. I can remember they didn't even have grass in the courtyard yet. But I don't know the politics that were involved in why Herb got old labs and not new labs. But most of the rest of the people in the microbiology department were in the new towers and there were teaching labs and the departmental office in the old building where we were.

We didn't have a lot of interaction with the other people in the department. We had a dishwashing facility in the new building, a room. We had some interaction with people in [J. Michael] Mike Bishop's lab.

Hughes: Because you were doing similar research?

Betlach: Well, it wasn't that similar. They were doing tissue culture and working on viruses, whereas we were working with bacteria. But they were just nicer to us. [laughter] What can I say?

Hughes: Do you know why Dr. Boyer was recruited?

Betlach: That was before my time, so I don't really know. I came in 1972.

Lab Personnel

Hughes: Who was there when you came in 1972?

Betlach: Not very many people--Joel Hedgpeth was just graduating and Ned Mantei whose job I filled. I came to take his position. I came in as a technician. Ned was going back to graduate school with Charles Weissmann. We both had previously worked for Hatch [Harrison] Echols at the University of Wisconsin. Let's see, Bob Helling, Daisy Roulland-Dussoix. That's all that comes to my mind right now.

Hughes: What was she?

Betlach: It was so long ago and there's been a lot of postdocs and sabbaticals and everything under the bridge since then. I think she was not a postdoc. I think she was higher than that. I think she was on sabbatical or she had some temporary appointment. Herb should know that. Bob Helling was definitely a sabbatical.

So there weren't very many people at first, but then six months after I was there, Pat Greene came in as a postdoc. And then other people started coming, like Paco Bolivar and Ray Rodriguez and Bob Tate and Herb Heyneker, and we started to really get crowded.

Hughes: This was when the research was heating up?

Betlach: The increase in people happened because, yes, we started to--

Studying Enzyme Restriction and Modification Then and Now

Betlach: When I first went there, I was purifying restriction enzymes, of which there were not very many known, and there are hundreds now. Bob Helling was isolating plasmids and running them on tube gels. Pat [Patricia] Greene came and started to set up a gel assay for our restriction enzyme purifications, which previously had been done on sucrose gradients, which was laborious.

Hughes: What does that change in technology mean?

Betlach: That was a big leap. Initially, restriction and modifications systems were investigated in vivo. For example, you could infect an *E. coli* strain with unmodified phage and you'd see if your phage titer decreased in comparison to control strains that either contained the restriction modification system or did not. The presence of the restriction modification system in the strain you were testing was indicated by a decrease in titer since the phage would be susceptible to digestion by the restriction enzyme.

For purifying restriction enzymes we used an in vitro assay. We took radioactive lambda DNA and we'd mix it in a tube with our enzyme preps that we were assaying. So you'd collect fractions across a given column during any purification step. There would be several different column purification steps and you'd have these fractions and you'd take a small amount and you'd react it with the radioactive lambda DNA and then run it on a sucrose gradient. So each sucrose gradient was one sample, one fraction we were assaying, so it was really labor intensive. The sucrose gradients were run in an ultracentrifuge, fractionated, and counted in a scintillation counter. A lot of work.

Today you take an enzyme sample; you react it in the tube the same way only the DNA is not radioactive--and then you run it in one slot on a slab gel. You can do forty at a time in half an hour, instead of all day to do six because an ultracentrifuge would only hold six samples.

Hughes: Did you have an ultracentrifuge by the mid-seventies?

Betlach: Yes.

Hughes: I know you didn't in the beginning.

Betlach: Yes, we had one and a cold room we used for enzyme purifications. I was trying to remember where our Sorvall was. I can't remember where it was.

Ernest Jawetz and His Lab Group

Betlach: I remember that sometimes we needed an extra Sorvall centrifuge and that across the hall in the department, the chairman had a Sorvall in his lab. But he was an old-fashioned guy, Ernie Jawetz. His Sorvall was absolutely spotless and never used and never touched and he would not let us use it. I got to use it sometimes because he thought that I was neater and cleaner than the rest. It was really sexist. [laughter] The Sorvall was actually under a plastic sheet. Here we were slaving across the hall, people lined up trying to use this other

piece of equipment, hopelessly overloaded. That's the kind of atmosphere; that probably tells a lot.

Hughes: Was there tension between the two labs?

Betlach: Sure. Not only between Herb and Jawetz, but also with the whole lab.

Hughes: You mean one lab against the other?

Betlach: Well, he didn't really have very many people in his lab. All I can remember him doing was walking around in his white lab coat. I'm trying to remember the names of the people involved in setting up the microbiology classes, people pouring agar plates and so on.

Hughes: There was a woman named Hanna.

Betlach: Lavelle Hanna. White hair, yes. And then there was another one with dark hair and glasses. I can't remember her name. I liked her a bit better. They were all sort of remote, that threesome. Not very friendly, and like old-time microbiologists from the '30s or something. Just a different generation.

Hughes: And working on chlamydia, weren't they?

Betlach: I don't even know what they were working on.

Hughes: That says something in itself about the lack of communication.

Betlach: Yes, I didn't even know what he was working on. It didn't seem to me like it was working. [laughter] He was just being the chairman. Low key. We were turned up a couple of notches from them. Some resentment there.

They did do some remodeling on one of the labs and made an office for Herb when I was there. They did do that for him.

Hughes: So the gist is that Dr. Boyer didn't feel particularly supported?

Betlach: I think that's probably true.

Hughes: A classical microbiologist might have recoiled when you mentioned molecular biology.

Betlach: But there really wasn't molecular biology back then.

Hughes: Why do you say that?

Betlach: I think of molecular biology as being more modern, using recombinant DNA technology and cloning and working on the molecular level. We weren't working on the molecular level at first. We were doing restriction modification studies *in vivo*, which was more similar to what Jawetz was doing. But then we were starting to leap forward, doing *in vitro* studies, getting more to the molecular level, see? And Jawetz wasn't doing that.

Hughes: How were you thinking of yourselves?

Betlach: What do you mean?

Hughes: What were you? Were you geneticists?

Betlach: Well, each person in that group had a different background. Bacterial geneticists and biochemists are probably what we thought of ourselves as. We were just doing what we thought was interesting. And you make the leap, and you have an idea, and you test it. As I said to you on the phone, I feel like the work that I've been doing almost at every time of my life has been inherently interesting.

It was really obvious when we started to clone DNA from other organisms--it was the first time that that was being done--that that was a large advancement. We didn't know all that was going to come out of it, but we recognized that it was important. Herb really early on was talking about cloning insulin. And I used to think, that's a little bit far-fetched!

And all kinds of jokes about cloning frog DNA. Are the bacteria going to croak? Are they going to be green? It was fun.

Atmosphere of the Boyer Lab

Hughes: Talk about the atmosphere of the lab. Was there interchange at all levels? Everybody working together and exchanging ideas?

Betlach: Within the lab it was terrific. It was incredible. I was in that lab as a technician. I didn't have a Ph.D. and I was treated as an equal. There was a free flow of ideas. It didn't matter what level you were at. There was just a really good combination of people and there was a lot of free interchange of ideas.

Any idea I had was equal to any idea Herb had or any idea that any postdoc had. It was a real exciting time.

Hughes: Were you working long hours?

Betlach: Yes. And weekends.

Hughes: Weekends, really? That's just what you did?

Betlach: Yes, right! The work was interesting. You'd come in to follow something through because you had an experiment that was in the middle of being done. You didn't even think twice.

I was married at the time and my husband was pretty mad about it. He wanted me to be home at five or six every night. I got divorced soon afterwards. He was sort of jealous that my work was so gratifying to me and that the intellectual atmosphere of the lab was so stimulating to me. It was an extremely interesting place to be.

Hughes: Was it unusual to have such free interchange regardless of who you were?

Betlach: Not real unusual. I think you could find it in a number of places. I always sought out that kind of environment, and I had had it in other places before I came to Herb's lab. In Jawetz's lab it would be unusual.

Hughes: When you moved to the Department of Biochemistry you found the same free flow?

Betlach: Sure. If it's a good lab, it will be that way. It could be a good lab and not be that way but then I wouldn't want to be in it. When I was at the University of California, Santa Barbara, in Ed Orias's lab, it was also that way, but we weren't breaking ground in quite the same way as we were in Herb's lab. And at Hatch Echols's lab at Wisconsin it was also that way.

Hughes: You felt that you were at the cutting edge?

Betlach: Yes, but I felt that at other times. As I said, I always feel like the work I'm doing is inherently interesting and is on the edge or is interesting to me, and that's enough for me.

Recombinant DNA Technology

Early Development

Betlach: The part where it made a difference was when we could see recombinant DNA technology was going to have these really broad applications. And that added another level of excitement until we started getting negative press. That was kind of bad.

It was exciting to think about what the applications were, the type of things we could do. Everybody was sending us DNA which we were cloning like mad once we finished cloning the *Xenopus* DNA. And so there was just an incredible variety of experiments going on and people contacting us from all over the world.

Hughes: Follow that through a bit more systematically, perhaps beginning in 1972 when you arrived. The first cloning paper was published in 1973,[1] so it appeared pretty close to your arrival.

Betlach: Yes. I came and I started to purify restriction enzymes. Bob Helling was there and he was running plasmids on tube gels. Herb had started this collaboration with Stan Cohen. They put together the idea of restriction enzymes, plasmids, maybe we can do something. Yes, that was a pretty exciting idea. Annie Chang and I both did that experiment. I can't remember if we cut it and they ligated it, but I know for sure that I screened the clones because I had the original DNAs for a long time and I remember doing it, and we had really crude ways of doing it. We had to work out methods for purifying plasmid DNA from these clones in order to characterize them. The technology has since advanced a tremendous amount.

Hughes: So you had to invent that methodology? It wasn't in the literature.

Betlach: Oh, yes. In fact, I still have some of our original procedural write-ups[2], and we kept actively improving them all of the time. When these procedures got out into other people's hands, they developed faster and faster. My feeling was once I got any given method to the point where I could get what I wanted out of it at a reasonable pace, I didn't want to spend any more time working on the methods. I wanted to move on and actually do the work.

I have a procedure handwritten by Herb, "The Betlach/Boyer Plasmid Purification Procedure." It's two-pages long and involves many time-consuming steps, such as using a flash evaporator for one sample. And now it's just a lot more streamlined and you can buy commercial kits to do this. It was just like the Dark Ages what we were doing. It was pretty exciting.

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[1] S.N. Cohen, A.C.Y. Chang, H.W. Boyer, and R.B. Helling, "Construction of Biologically Functional Bacterial Plasmids *In Vitro," Proceedings National Academy of Sciences* 1973, 70: 3240-44.

[2]See appendix.

Authorship of the First Paper

Hughes: Why wasn't your name on the paper?

Betlach: Well, Herb has said a lot of times since then that my name should have been on that paper, and I think it probably should have been because certainly I made at least as equal a contribution as Annie Chang did. I have to say that I am a different person now than I was then, and I didn't think then to speak up to say, "My name should be on this paper." Whereas now, I would. This was unfortunate for me, but I was pretty young and naive.

Hughes: It wouldn't stand out if Annie Chang's name wasn't on the paper.

Betlach: I know. I was just trying to remember how long I had been there. Maybe at that time I was fairly new and Herb couldn't predict how much of a contribution I was going to make and how dedicated I was going to be.

Hughes: Well, that paper had to have been published before June of 1973 when Dr. Boyer talked about cloning DNA at the Gordon Conference [on Nucleic Acids].[1]

Betlach: I started in '72, in the fall, so it could very easily be what I said.

Hughes: That you may not have been in the lab for more than just a few months?

Betlach: Yes. But I did do--

Hughes: You did the work.

Betlach: Yes, it also may have been what I said. And as you said, the omission wouldn't be so glaring if Annie Chang also wasn't on there. And also if my contributions since then hadn't been what they were.

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[1]The first paper on cloning was published in November 1973.

Contemplating Commercial Applications

Hughes: Were the potential commercial applications of what you were doing immediately apparent?

Betlach: Well, Herb kept saying, and I can't put a date on it, "We can probably clone insulin." Or, "This could have benefit for society." So I have to say he was probably thinking that way. I wasn't. I was just enjoying the pure science.

Hughes: But it wasn't clear for some time that you could actually express proteins, was it?

Betlach: Yes, probably not. But I'm telling you, Herb was saying insulin really early. You can ask him and I'd be curious if he could remember exactly when, but I just know he was thinking about that long before anybody else.

Although if you think, okay we can clone human DNA, fly DNA, whatever it is, in bacteria, it is not a big conceptual leap to expression. You would think, maybe we can express it; we can grow a lot of bacteria and they'll be little factories. Although we didn't even know that much about promoters or regulation of expression then!

Cloning Eukaryotic DNA

Hughes: Well, do you want to talk about the frog [*Xenopus laevis*] work? It did cause a stir.

Betlach: Well, yes. I can remember it fairly clearly. I can remember what room I was in when I did it, of all the three labs. In our tenure there, I moved around to various rooms and I can remember that there weren't very many people there yet. And for some reason I can remember what the plates looked like, probably because it was a unique experiment at that time and it was done differently than other experiments. I remember handling the clones and making the DNA, I remember pretty clearly. I guess that says that it was different. Because that was a long time ago.

Hughes: Was there excitement when you saw that it actually did work?

Betlach: Sure! Yes. [laughs]

Reconstructing the Experiments

Hughes: Talk more about the technical parts of it. How did you single out the clones that actually had inserts?

Betlach: What we had to do was make plasmid DNA from the clones-- This reminds me a little bit of those depositions that I've been at when they're asking me to reconstruct experiments. [laughter]

Hughes: Sorry about the bad memories!

Betlach: No, not bad memories. It's just that I've done a lot of experiments since then. I'm just trying to reconstruct and I'm sure there's some gaps, but I can remember we made plasmid DNA. We grew up a liter of each clone. A liter, you know? Now we grow a couple of milliliters. It was horrendous.

Hughes: Why so much?

Betlach: Because there was really no method for doing it and we were trying to work out a procedure. Also, we thought that the DNA could be used as substrate for the restriction enzymes that we were purifying. One of Herb's major interests at the time was in characterizing the *Eco*R1 restriction enzyme, and these plasmids could be used as a substrate for the enzyme. But gee whiz, one liter and you have enough DNA forever. [laughter]

Actually that became apparent to me sooner than it did to Herb. Because I remember grumbling and thinking, we should grow up less. He kept insisting, "We can use this as

substrate for the enzyme." So I was already thinking, before him, that this procedure has got to be scaled down because it was in my hands and it was really horrendous.

Now what else was I talking about? Making the DNA. You purify the DNA and then it would have to be run on the gel and stained. We were doing these polyacyrlamide tube gels and they'd have to be stained.

Hughes: Why tube gels?

Betlach: Now, Bob Helling was the one that set that up in our lab. And he was doing it to characterize the plasmids found in different bacterial strains, and I think he published some papers on that work. I'm just trying to remember how that apparatus developed. Maybe tube gels were developed for some other purpose and he adapted them.

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Betlach: I remember when Pat Greene came, she established an agarose method in our lab. We were trying to figure out at first how to take photographs of these gels. Now companies sell special equipment for this. We were going down to the UCSF photography department and trying different filters and lights so we could get good pictures and documentation of the gels. We were having to work all of that out every step of the way. But that's the way science is. Now, it just seems like the Dark Ages because these things have evolved so far. Most of the time I don't think anything about it, but other times I just feel like some old fossil. I was one of or <u>the</u> first cloner. And here I am still doing it.

Hughes: What kind of reaction did you get from the scientific community?

Betlach: At what stage?

Hughes: Well, the way I read it, and I would like your opinion, is that DNA cloning wasn't making too much impression on the science community until the Gordon Conference.

Betlach: When exactly was that? I didn't go to the Gordon Conference.

Hughes: That was June of 1973. Dr. Boyer had an agreement with Dr. Cohen that Dr. Boyer would not say anything until the paper was published, which was in November 1973. In the enthusiasm of the moment, or whatever--I don't know what his motivations were--he did talk about it. It fell flat until somebody at the Gordon Conference picked it up and then the implications became clear. A result was the Singer-Söll letter.

Reporting the Discovery

Hughes: Do you remember talk about keeping the research quiet until it was published?

Betlach: No, I can't remember about that specific instance, but I can probably shed some light on why it might have happened like that and on Herb's personality, which might help you. I don't like my postdocs to talk about work unless it's actually been submitted for publication. If it's submitted, it's okay. It's better if it's accepted, but at least if it's submitted, if they're going to reject it, at least we've got something that we can rework and send back. But some labs have policies that you're not supposed to talk about work until it's published. So it sounds like there was a difference in opinion between Herb and Stan Cohen.

Herb is the kind of guy that never held anything back. He's not as uptight as I am about that. As soon as something happens, he doesn't care if it's written up, he wants to talk about it. This can be a disaster, but on the other hand it really helps the flow of scientific ideas. Also, Herb was not really good about getting things written up. [laughs] Stuff would end up being written up in weird places, proceedings of meetings and stuff like that. I realized, he would rather do his science and not the writing.

Hughes: Once he wrote it up, he didn't necessarily put it in the best journal?

Betlach: No. He didn't really care. Some of our stuff was published in the weirdest, funkiest places. Some of the stuff should have gone to really premiere journals, and didn't. Now, years later, if I publish work in meetings proceedings, it's usually review-like in nature and

contains little really new data. I don't think it's that Herb didn't feel confident in the work; I think it's just that he didn't like to write that much.

Hughes: Well, the 1973 paper was published in the *Proceedings of the National Academy of Sciences*.

Betlach: Well, that's good.

Hughes: Why would they have chosen that journal?

Betlach: That's a good journal and it has a really wide readership and it's an appropriate place. So that indicates they knew it was important--Stan Cohen, I'd say. I just feel Herb doesn't control his contributions into the literature that much. He likes to got to meetings and talk about it and tell people about it. He likes to do the science. I bet that was Stan Cohen's decision. But don't tell Herb I said that. [laughs]

Hughes: Are there differences in scientific styles between the two men?

Betlach: Yes. Stan Cohen is a little more tense and controlling. We had Herb's retirement party a couple of years ago, and Stan came to it and it was really nice. A lot of people came to it. It was really fun.

Hughes: They don't see each other very much now?

Betlach: No.

Hughes: Did the collaboration end after the cloning work?

Betlach: Not much happened after that. Maybe some minor things, but nothing that I can remember specifically.

Biosafety Concerns

Hughes: Relating to the recombinant DNA debate is a paper by Dr. Cohen stating that transfer of DNA occurs under natural circumstances. This was supposed to allay the fear of breaching species barriers and the potential hazard of recombinant DNA research.

Betlach: Yes, and that transfer does occur.

Hughes: Was it a big deal that he showed experimentally that it could happen?

Betlach: I can't exactly place that piece of work to this time. But we were not worried at all at first, and then it became clear that maybe there might be a reason to worry.

Hughes: When and why?

Betlach: There was the meeting at Asilomar [February 1975]. I remember, I was doing a lot of cloning of DNA from all kinds of organisms. [laughs] It was like the zoo. We just put them on the shelf; we just quit; we just stopped. I can't tell you exactly when.

Hughes: You're not sure that it was after Asilomar?

Betlach: I think, if anything, it might have been before. As soon as there was any clue at all, before that meeting. But I just can't recall exactly. I'd have to look through my old notebooks, which I no longer have. Three years of my notebooks disappeared at some point.

Hughes: Really.

Betlach: And I am very careful about my notebooks. I gave a lot of them to Herb when I left UC. In some of the patent contests that have been going on between Lilly and Genentech and UC, I was deposed a couple of times. The last one I was up for, they asked me, "Do you know what happened to your notebooks between the years 1976 and 1979?" I think those

were the years. And they're just gone. There was an earlier stage when I was giving testimony when they were there, and large chunks of them were copied by various people involved.

Hughes: But you got them back?

Betlach: I got them back after parts of them were copied, and then they subsequently disappeared. I never lose anything like that. I have every single notebook I ever had, except for those that were lost and the ones I gave to Herb.

You asked if we were aware of the possible dangers.

Hughes: Yes. Can you recreate the feeling of that time?

Betlach: Were we really worried, you mean?

Hughes: Yes, and how did you react to the dissenting scientists and the environmental groups that were activated on this subject.

Betlach: Well, I can only speak for myself. I felt that there probably wasn't any danger, but I didn't know, okay? Especially human clones, you just really didn't know. So we put them on the shelf. Probably it would have been better to autoclave them.

When we got so that we had to certify vectors and things, we went through some really tedious testing, putting them in crippled bacterial strains, to make sure that there wouldn't be any danger. And if you sat and thought for a couple of minutes: these genes are in bacteria. Are you going to inhale them? Or maybe you're going to get them in your gut. Your body's going to have defenses against these kinds of things and probably nothing is going to happen.

We were aware that there might be mechanisms that we didn't know anything about, so we were as careful as we could be. And I think it was overkill. Even then I was thinking what we had to do and all of this furor about P3 [physical containment lab level 3]. I was

far more afraid of working with hepatitis virus or Rous sarcoma virus, like Mike Bishop was working with at the time. Tissues from diseased patients, I think, were much more dangerous.

Hughes: So you weren't particularly afraid for yourself?

Betlach: No, I wasn't worried. But I was young and did all kinds of things in those days, like not using gloves when using ethidium bromide and using huge amounts of P_{32} [radioactive phosphorus]. Now I wouldn't do such things.

Hughes: It wasn't an awareness in that era?

Betlach: I wasn't the only person. [laughs]

Hughes: It was standard behavior, is what I'm asking; it wasn't that you were a risk-taking person?

Betlach: No. Ethidium bromide, I just didn't know; I thought it was okay. When I learned otherwise I started wearing gloves. Radioactivity I should have probably known better and times change and people use less radioactivity in experiments now. But these clones--I didn't think there was any danger. But I didn't know for sure. I remember clearly having this feeling, especially with human DNA: there may be some mechanisms I don't know. Okay, we'll just put these away, and we've got plenty of other things to work on. And we did. That's my personal feeling.

Hughes: Do you know for what period they were put on the shelf?

Betlach: Oh, for a long time. We completely shelved a whole bunch of experiments. We had been sent DNA from people to do experiments with. When specific experiments with human DNA, for example, started up again, they were P3, and each experiment was usually assigned to one postdoc. It wouldn't be just me slinging hash on six things at once. I was much more focused on making vectors by that stage. By then we'd send vectors out and the recipients would be doing the cloning experiments themselves in their own labs.

And then we had a couple of projects where Herb thought that he was going to get interested in immunology. I remember him saying, "Immunoglobulins are going to take up the next twenty years of my life." We had a couple of postdocs who were starting to work on that, and so I just didn't work on any of those experiments anymore. I started to make vectors and improve vectors, which was kind of fun.

Hughes: When you were thinking about risk, what exactly were you thinking might happen?

Betlach: I didn't know. I was a bacterial geneticist. At that time I didn't know anything at all about eukaryotic systems, except in very gross terms. In fact, not that much was known at the molecular level on human systems. Period. However, I was afraid of Rous sarcoma virus and hepatitis virus. I just thought, I don't know about this, and there may be mechanisms that we don't know about and it's just not worth it. I wasn't terrified by it. I recognized that we didn't really know all that was going on. Actually, if there wasn't a lot of fuss about stopping it, I probably would have just done it, but it would have made me a little uneasy.

Hughes: Presumably you started again when the NIH recombinant DNA guidelines were weakened and such experiments were now permissible.

Betlach: Yes, but that's the period when I said that I was focusing on something else besides cloning the DNA everybody was sending. Individual postdocs had projects where they would work with one sample of DNA. At the early stage, before the Asilomar meeting, we were the only place that was doing it. So people were sending us DNA, and we were cloning all kinds of things. Later, we started to focus on interesting new projects that would use this tool.

Plasmid Vectors

Plasmid Development in the Boyer Lab

Hughes: Well, talk about vector development.

Betlach: Sure. Now let me shift gears. Of course, we wanted to develop vectors that would be widely useful. I know that's what Paco Bolivar and Ray Rodriguez wanted to do. My feeling was, I want to make a vector I can use to do things I'm interested in. I don't want to make vectors for the rest of the world.

Hughes: They were in the Boyer lab to make vectors rather than to pursue their own research interests?

Betlach: Many people who joined the lab at this time came to learn the new technology. I don't know the specifics reasons they came for. When they arrived I was already developing new vectors, and then they took ones I had made and refined them more.

Hughes: Go into exactly how one does that. Do you want to use pMB9 as an example?

Betlach: What they wanted to do was to make the plasmid easier to use, so it would have more general utility. Put more antibiotic resistance markers on it, so that you could clone in one antibiotic-resistant gene and inactivate it and select for the other marker. We could already do that with pMB9, which has the markers tetracycline resistance and colicin immunity. However, colicin immunity is a tricky marker to use.

They wanted to use ampicillin resistance and then the idea was you would clone in, for example, the tet gene and your transformants would be still ampicillin resistant. But they'd be tet sensitive and then you'd have a way to tell which clones were which without looking at the DNA. So it's actually a screen. And they wanted to make the plasmid smaller, take it down to the basic elements so you have less DNA there, and make maps of it so you know all the sites in it. Then Greg Sutcliff sequenced the entire thing, pBR322.

Hughes: What about sequencing techniques at that time?

Betlach: Primitive, primitive! John Shine was doing some and Joel Hedgpeth did some too. It was primitive and very difficult compared to now, just like everything else we were doing then.

But the way we got started on doing vectors was we had originally pSC101 which came from Stan [Stanley N.] Cohen's lab that was tet resistant and did not have very many copies per cell, so you didn't get a lot of DNA. It was big and difficult to use.

Meanwhile, the problem I was interested in was, I wanted to clone the *EcoR1* endonuclease genes onto a multi-copy plasmid. Hopefully then it would be expressed at higher levels. We'd have an over-expressing strain so when we made restriction enzymes we'd get a lot more enzyme. I tried to isolate and clone the *Eco*R1 endonuclease and methylase genes, and it turns out, they were already on a multi-copy plasmid. Actually, that's an interesting story, too.

Isolation of *Eco*R1 and the Development of Betlach Plasmids

Betlach: *Eco*R1 was originally isolated from a clinical isolate from the UC labs. You can ask Herb about this. It was isolated from someone's urinary tract infection. [laughs] Multiple-drug resistant *E. coli* organism. A student of Herb's named Bob Yoshimori got that strain and worked on it first a little, and then left.

So I took that strain and was trying to clone these genes from it. I characterized it and we found, as I said, that the R1 genes were already on this multi-copy plasmid. And then I cloned the methylase gene. That was probably the second cloning experiment done anywhere. This plasmid, it turns out, was multi-copy, and then we also had pSC101, this large low copy number plasmid from Stan Cohen which was difficult to use but that had tet resistance on it.

The first plasmid that I isolated from the clinical isolate I named pMB1. It was big too, but it was multi-copy, so we tried to get it down a little bit in size. We didn't have very

many restriction enzymes at that time, so I took pSC101 and pMB1 and did an *Eco*R1 star digest which is a decreased specificity for the *Eco*R1 site. This probably all means nothing to you. It was like a witch's brew, okay? We didn't have a lot of tools to work with and I just sort of mixed things together, selected for tet resistance, and hoped for the best. Out came a bunch of clones, one of which I named pMB9, that contained the tet gene from pSC101, but the origin of replication from the multi-copy plasmid, pMB1.

Hughes: Do you do the procedure very deliberately so that you know that you're going to get the tet gene?

Betlach: Not exactly. I selected for tet resistance, but a fairly random mixture of fragments was used. There were not very many restriction enzymes and no restriction maps yet. There was *Hind*3 and there was *Eco*R1. If you react *Eco*R1 under certain conditions, you get a decreased specificity, so it hits in more places. But it's almost random, so I digested a mixture of pSC101 and pMB1 with this fairly random-cutting enzyme. I put a selection on for tetracycline resistance. I selected for that so it would pull a little fragment containing tet resistance out of the mixture. I always felt like it was a witch's brew, and I guess I was the witch. [laughter]

I remember I got this set of clones and I ran them out on a gel and there was only one that looked any good. I thought, oh, I can't wait to check that one out, and I went on vacation for a month because it was in the middle of the summer and I had already made these vacation plans and I couldn't find out the result until I got back. I came back and then I characterized it and the one promising clone (which I named pMB9) was good for a lot of things. I could do a lot of things with it and so that's why I stopped developing it. It was small, it was multi-copy, it had tetracycline resistance on it. It was useful. And we gave pMB9 out to a lot of people.

Plasmid Dissemination

Hughes: What is the protocol in science for exchange of materials?

Betlach: Well, usually once you publish information on a strain or plasmid etc., you're morally obliged to give it out. Before publication, you're really not. In fact, some journals stipulate that you have to give out anything that's published in their journal. Not everybody does, but we freely gave out all sorts of materials. We gave out pMB9 all over the world before anything was published on it. It didn't bother me. I didn't care because I wanted it to use it for experiments and it was fine. And if it's useful for other people's purposes that aren't in competition with you, then why should it bother you? So we gave it out all over the place.

Hughes: Were there any restrictions?

Betlach: None that I can remember.

Hughes: Did you stop distribution for a while when there began to be the concern about recombinant DNA?

Betlach: Well, we had pMB9, pBR313, and pBR322, and they were being tested. I think we did stop sending them out until we got the plasmids certified. Or I may have given them out to some people, but told them that we were waiting for certification and they couldn't use them before we got it. I can't remember exactly, but I think it makes sense that we just stopped sending it out.

Hughes: That leads into the pBR322 episode. My main source is Stephen Hall's book, *Invisible Frontiers*.

Betlach: That one is pretty good. Accurate.

Hughes: Yes. *The Race to Synthesize a Human Gene* is the subheading. He maintains that Dr. Boyer in January of 1977 was very clear on the distinction between an "approval" and "certification."[1] Remember that two-step process?

Betlach: I remember it. It's very clear in my mind.

Hughes: It apparently was not clear to other people in the department.

Betlach: That may have been the case.

Hughes: Namely, to Howard Goodman and William Rutter.

Betlach: Well, I don't know if they were clear on it or not, but I definitely knew that it was a two-step process. People were calling me up all the time. I remember Herb was down in Miami or someplace at a meeting, and the plasmid was at NIH and they were deciding it had been approved and not certified, or certified and not approved, now I'm temporarily confused.

Hughes: "Approved" comes first. Certification was by [Donald] Fredrickson, the NIH director.

Betlach: What's the name of the other guy, Gartland?

Hughes: William Gartland. He was head of the NIH Recombinant DNA Advisory Committee.

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[1] Stephen S. Hall, *Invisible Frontiers: The Race to Synthesize a Human Gene*, (Redmond, WA: Tempus Books, 1987), pp. 116-17.

The Boyer Lab Moves to the Biochemistry Department

Hughes: What difference did you find between the Department of Microbiology and the Department of Biochemistry when you moved at the end of 1976?

Betlach: When we moved up to the fifteenth floor in the Health Sciences Towers?

Hughes: Right.

Betlach: Well, we had a giant new lab, and that was great, but we were up on the fifteenth floor and we were fairly isolated at first. For me it wasn't really that much different because we interacted just as much with people in the Biochemistry Department [ninth floor] when we were in the Microbiology Department. There was actually quite a bit of cross-talk between Microbiology and Biochemistry. The departments overlap a lot now. They have the PIBS program--Program in Biological Sciences; the graduate programs are shared in a lot of ways. But it was nice to be away from that oppressive Microbiology Department feeling. But otherwise I'd say the interaction with Biochemistry stayed the same. And we had these gorgeous labs.

Hughes: You didn't have more collegial support?

Betlach: We already had a lot of collegial support from the Biochem department. So, I think it was the same. [William J.] Rutter did an incredible job of building up that department. And there's been a lot of excitement and development into other scientific areas since then. The UCSF Biochemistry Department is ranked first or second in the nation.

Use of an Uncertified Plasmid, 1977

Hughes: Well, we were talking about the pBR322 episode. You said you were very clear on the distinction between "approval" and "certification."

Betlach: Yes, I knew that we needed both. People would call me up. A lot of times I'd be busy and they'd ask me if pBR322 was okay to use, and I'd say, "It's approved, it's approved!" Not necessarily would they hear that you need both "approved and certified." So you could see people might take my answer the wrong way.

Hughes: All of this was verbal at this stage?

Betlach: Yes, phone calls.

Hughes: What about the insulin clones?

Betlach: All I know is hearsay. I know that people were calling me up and I told them what I told them. Everybody had the plasmids because we were so freely giving everything out. You're asking me whether or not the plasmid was used ahead of NIH certification?

Hughes: Yes.

Betlach: I can give you an opinion. People could have misunderstood what I told them on the phone and could have used pBR322 before it was certified. One could imagine a lot of scenarios. And then, like you, everyone is saying this, that, and the next thing. All I can tell you is my personal feeling and I don't really have anything to substantiate it: Probably pBR322 was used and probably used by mistake because there was a misunderstanding.

Hughes: And then destroyed? Once it was revealed that the plasmid had been used before certification, Axel Ullrich supposedly destroyed the clones.

Betlach: Yes, but the problem with that was the experiment had to be redone.

Hughes: Some people didn't believe that the experiment could be repeated and submitted to *Science* within three weeks. They questioned whether the original clones had been destroyed.

Betlach: The experiments were pretty quick to do and Axel would have all the materials to do it. He'd have the RNA. This is a guy that worked night and day.

It was really sort of an incestuous situation at the time. All of the postdocs in the department were socializing. So you were living with these people all the time, and there'd be big parties, and we partied pretty heavily. All of this was going on. I knew Axel pretty well. He was dating my roommate at the time. My marriage had broken up and I was sharing an apartment with another divorced woman.

I remember when this other woman and Axel and I watched this *Nova* show on TV, recreating all of this. We just sat there, silent. [laughter] It was really–

Hughes: Tense?

Betlach: Not tense. Just quiet, thoughtful.

Hughes: Was the program accurate?

Betlach: I can't remember how accurate it was. I just know we each knew our own truths, if you know what I mean.

Hughes: You didn't ask, "Axel, did you destroy the clones?"

Betlach: No, I did not because by that time I think Bill Rutter had been sending around little notices to everyone saying, "What do you know about this?" Because he was trying to figure out what went on, maybe before going to the Senate hearing. I didn't reply because I felt I really didn't know anything, except hearsay and the fact that when people called me I told them X, Y, and Z. So I wasn't going to contribute to the general paranoia and weirdness that was going around, so I never talked to Bill Rutter about it.

Hughes: Well, I saw Dr. Ullrich at the Rutter symposium, and I asked him if he would talk with me.[1]

Betlach: His future wife Suzanne also socialized with us.

Hughes: What lab was she in?

Betlach: She was a graduate student.[2] Whose lab was she in? Reg[is] Kelly's? And now Axel's at the Max Planck Institute in Munich. I was there at a meeting last spring and I stopped by his lab, but he was in Spain. I just left him a little note. I haven't seen him in a long time. And the little note I left said, "Here's a blast from your past." [laughter]

Rivalries

Hughes: There was quite a bit of intra-departmental rivalry at that time, for instance, between Goodman's group and Rutter's group. There was also Harvard.

Betlach: Oh, yes, Harvard. I remember we felt the competition with Wally [Walter] Gilbert's group at Harvard. They put out these little newsletters "The Midnight Hustler,[3]" talking like we were sports teams or something. Those competitions were all very good-natured.

Hughes: Even within the department?

Betlach: Well, Herb and Howard had a falling out. We never had any problem with Bill Rutter, and I have a very high opinion of him. Any problems there were between him and Howard, I don't know much about. But I know Herb had a falling out with Howard. We had a lot of interaction with everybody in Howard's lab, up to a certain point in time. In fact it was almost like the two labs were one lab. We had joint seminars and we shared supplies. There was a lot of camaraderie.

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[1] An interview with Dr. Ullrich was conducted on April 5, 1994 and will be available after a second interview is recorded.

[2] Subsequently, she got a faculty position at Stanford. [MB]

[3] See Appendix H.

UC's Contract with Genentech on Somatostatin

Hughes: Did you have any part in the work on somatostatin?

Betlach: I had a more peripheral role in somatostatin. At this time Herb had been talking to Bob [Robert A.] Swanson. Genentech had been formed and they wanted to start doing some work. Somatostatin was one thing they wanted to start working on and a contract was set up somehow--all kosher with the university. There was a little bit of money that was made available to the lab to do this.

Hughes: From Genentech?

Betlach: Yes. And so, I was put on it at first, along with Art Riggs. He came up from southern California. Art Riggs didn't know much molecular biology at the time, and so I had to teach him. We were doing the somatostatin research together. He sort of drove me nuts.

Hughes: Why?

Betlach: Well, he's one of these real methodical guys. He's too slow. I was very impatient with him, and I'm not very good at teaching, either. So we started to work on it for a while and then I got off the project. Actually, I remember it fairly clearly because I was deposed for this pretty recently and they went through all the experiments. "Do you remember you did this experiment, etc.?" but that's about all there is to it that would probably be of interest to you. My role was more peripheral.

Hughes: Congress at the time was debating whether legislation should be passed to regulate recombinant DNA research. Which, of course, the scientists--

Betlach: Did not want.

Hughes: The cloning of somatostatin was announced in a Senate subcommittee hearing. The point was to show the commercial and medical possibilities of this technology and allay some of the scare talk. Do you remember--?

Betlach: No, I don't remember much about that. All I know is Rutter was collecting data points from people before he went to the Senate hearing. I was talking to Herb every day, and he didn't discuss with me any strategy about what he was going to say or what they were going to do.

Hughes: Did you get the feeling that they were preparing for the hearing?

Betlach: Just Rutter.

Hughes: But not Dr. Boyer?

Betlach: I didn't have any feeling that he was. But I don't know. Herb has this laid-back style, so he may be preparing for something and you don't have a clue. [laughter] That's the way he is.

Boyer as a Lab Director

Hughes: How true is that laid-back style?

Betlach: It's true, but he's wonderful to work with because he's really intuitive. When he was interested in science it was wonderful to have him around because he's really intuitive and he allows people working under him to have an incredible amount of independence and encourages it. And he encourages you to be intuitive, too! So there's these wonderful ideas bouncing back and forth. But he's very laid back. He's not going to come and tell you how to do things. I've heard it said about him that he gives you enough rope, just don't hang yourself with it. I functioned really well in that environment because I never could stand somebody breathing down my neck. Stan Cohen seemed a little bit more like that. However, with Herb it was take the ball and run.

Hughes: How did his laid-back style work with graduate students?

Betlach: It depended upon the graduate student. Actually, he didn't have that many graduate students. Postdocs did better--they are more independent. Postdocs thrive in that kind of

environment, but for graduate students it's a little tough. Usually they floundered, unless they were taken under a postdoc's wing.

Hughes: Dr. Boyer told me he didn't like teaching medical students.

Betlach: Yes, he didn't like giving formal lectures to medical students, but he was really good at teaching postdocs in this indirect way because he encourages independence.

Tension over University Ties with Genentech

Hughes: One more question as I know I'm taking a lot of your time: Talk about the controversy that grew up in the early days of Genentech when it was functioning in the biochemistry department.

Betlach: Bad feelings. There were a lot of people in the department looking down their noses at Herb, saying that science and industry should be separate. He was blacklisted by a lot of people in the department, to be perfectly frank. Finally, I think, they've come around and they're all involved in new companies now. But it was pretty bad and pretty crummy treatment of him. I thought Genentech was a pipe dream, to tell you the truth. I thought it wouldn't go anywhere. But it seemed to me, if Herb followed all of the rules that he had a right to try something like that. If he was fulfilling his obligations at the university, nobody had the right to any say about it. That's what I thought.

Hughes: And he was fulfilling these obligations?

Betlach: And he was. It was really irritating to me that these people had this attitude. I continued to be irritated about it for the next twenty years after that. Some of them stayed that way for a long time.

Hughes: How was it expressed?

Betlach: A lot of it indirectly.

Hughes: Was Dr. Boyer cut out of scientific interchange?

Betlach: No, people that were interested in the same things that we were would still interact with us. No, it was more people that were working in other areas of research. All I can remember is disapproving looks, and I don't know specifically what things were done or said to Herb. I do know some, I'm sure, but I can't come up with any specific instances right now that can properly convey the feeling that there was.

Hughes: How did he react?

Betlach: Well, it wasn't fair. I remember I was hurt. I'm not exactly sure if he didn't give a damn or if it bothered him. Some of the people he really didn't think that much of, anyway. So if they were going to have that opinion, it was their problem. But then maybe there were certain individuals who felt that way, and maybe it bothered him, so probably it was dependent upon the individual.

Hughes: Does he talk about it?

Betlach: No, he doesn't. I've known him for a long time and I feel like I know him pretty well. He's laid back, but he keeps a lot of things inside. I don't think very many people know him very well, and I feel like I'm probably one of the people who knows him the best, and I don't know him very well.

Every year when Nobel Prize time comes around, I feel upset for him. I think now he's gotten over a lot of this because he's doing so many different things in his life now. About three months ago I finally sat down and told him how I felt, that I felt like it wasn't right, that I felt like he should have the Nobel Prize. A lot of these things had been unsaid and I just said them.

Hughes: How did he react?

Betlach: I think he appreciated the support, but he wasn't forthcoming. I could see how he felt from his body language and knowing him, rather than by what he was saying. It's easy to scratch

the surface with him but hard to go deep. So I can see how it would be easy to interview him but difficult to get some things out of him. Kary Mullis got the prize for PCR [polymerase chain reaction]. Herb Boyer should have the Nobel Prize. I think Paul Berg deserved the Nobel Prize; I don't know if it should have been for recombinant DNA. I think for recombinant DNA and cloning, Herb deserves the Nobel Prize.

Hughes: Is there anything more you want to say?

Betlach: I think I've said a lot. I don't know what else to say, except I'm glad I was there. It was fun.

Transcribed by Michael J. McArdle
Final Typed by Julie Allen and Kathy Zvanovec-Higbee

TAPE GUIDE--Mary C. Betlach

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POSITIONS/EMPLOYMENT

2001-	Director, Grant Research Collaborations, Sunesis Pharmaceuticals, SSF,CA
1993-	Adj. Associate Professor, Pharmaceutical Chemistry, UCSF
1999-2001	Director, Scientific Operations, Kosan Biosciences, Hayward, CA
1995-1999	Director, Molecular Biology, Kosan Biosciences, Hayward, CA
1993-1994	Sr. Scientist, Cell Genetics, Parnassus Pharmaceuticals, Alameda, CA
1983-1993	Research Specialist, Biochemistry & Biophysics, UCSF
1972-1983	Staff Research Associate, Microbiology, Biochemistry & Biophysics, UCSF

EDUCATION

1963-1967	Undergraduate (Biochemistry), University of Wisconsin, Madison, WI
1992	Ph.D. (Biochemistry/Molecular Biology), UCSF

HONORS/AWARDS

1963-1966	University of Wisconsin Scholarship
1967	University of Wisconsin Leadership Scholarship

EDITORIAL AND GRANT REVIEW PANEL SERVICE

1981-	Ad Hoc Research Grant Reviewer for National Science Foundation.
1983-	Ad Hoc Editorial Reviewer for *J. Mol. Biol.*, *Nucleic Acids Res.*, *J. Bacteriol.* and others
1986,1987	FASEB LSRO (ONR) Grant Review Panelist: Archaebacteria
1987	FASEB LSRO (ONR) Grant Review Panelist: Molecular Marine Biology
1990	Ad Hoc Research Grant Reviewer for Canadian Research Council
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PATENTS

1977 Expression of Heterologous Polypeptides in Halobacteria, U.S. Patent # 5,641,650.
2000 Sorangium Polyketide Synthase, U.S. Patent #6,090,600.
2000 Recombinant Narbonolide Polyketide Synthase, U.S. Patent #6,117,659.
2001 Recombinant Oleandolide Polyketide Synthase, U.S. Patent #6,251,636.
2001 Production of Polyketides in Plants, U.S. Patent #6,262,340.

2001 Sorangium Polyketide Synthases & Encoding DNA, U.S. Patent # 6,280,999.
2001 Six pending patent applications on various aspects of combinatorial biosynthesis and polyketide production.

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December 1972

CURRENT RI ENDONUCLEASE PURIFICATION PROCEDURES

For 300 gm wet-packed cells (RY 13). All steps at 4°C.

1. Suspend in 1 L EB and sonify.

2. Centrifuge for 70 min, 35,000 RPM, Beckman 35 Rotor.

3. Add 300 ml fresh 5% streptomycin sulfate (in H_2O), centrifuge low speed, 30 min.

4. Add an equal volume of cold saturated $(NH_4)_2\ SO_4$ (in H_2O) and stir for 30 min. Centrifuge at low speed.

5. Suspend in 300 ml EB and dialyze for about 3 hr against 4 L of EB.

6. Apply immediately to PC column (2 X 60) [4 x 35] equilibrated with 0.2 M NaCl EB. Run 0.2 to 0.6 M NaCl EB gradient total volume of 4 L. [2.5]

7. Pool fractions with RI endonuclease activity (which elutes around 0.4 M NaCl EB. At this stage the RI endonuclease will aggregate (presumably because of its hydrophobicity) below NaCl concentrations of 0.2M.

8. Dialyze the pooled RI endonuclease fraction against 0.2 M NaCl EB.

9. Apply to hydroxyapatite column (2x20 cm) equilibrated with 0.2M NaCl EB. Run $K\text{-}PO_4$ (pH 7.0) gradient of 10-400 mM in 0.2M NaCl. Endonuclease activity elutes around 0.25 M $K\text{-}PO_4$.

SKIP, PUT TRITON IN POOL

10. Dialyze against 0.2 M NaCl EB + 0.15% triton X, then against EB + 0.15% triton X.

11. Apply to DE-52 column (0.9 x 10) equilibrated with EB + triton X and elute with a NaCl gradient of 0 - 0.3 M, total volume of 1 L. The RI endonuclease elutes about 0.15 M NaCl.

12. Dialyze against EB-triton X and concentrate on a small DE-52 column. Elute with EB-triton X-0.3 M NaCl. Dialyze against EB-0.3 M NaCl. Store at 4°C.

December 1972

CURRENT RII ENDONUCLEASE PURIFICATION PROCEDURES

For 300 gm wet-packed cells (RY 22). All steps at 4°C.

1. Suspend in 1 L EB (10 mM K-PO_4, pH 7.0, 1 mM EDTA, 7 mM mercaptoethanol) and sonify.

2. Centrifuge for 70 min, 35,000 RPM, Beckman 35 Rotor.

3. Add 300 ml fresh 5% streptomycin sulfate (in H_2O), centrifuge low speed, 30 min.

4. Add an equal volume of cold saturated $(NH_4)_2SO_4$ (in H_2O), stir 30 min. Centrifuge low speed.

5. Suspend in 300 ml EB and dialyze against EB (usually 8 L overnight).

6. Apply to Whatman DE-52 (DEAE-cellulose) column (5x60 cm) in EB. Gradient of 0-0.5 M NaCl in EB, total gradient volume of 4 L.

7. Pooled fractions with endonuclease activity has 0.1-0.14 M NaCl.

8. Dialyze DE-52 pool against EB.

9. Apply to phosphocellulose (1 x 20 cm). Wash with 0.2 M NaCl EB and run gradient of 0.2-0.5M NaCl in EB; total volume of gradient is 1 L.

10. Pooled endonuclease activity has NaCl concentration of about 0.26-0.30 M.

11. Dialyze against EB and apply to (2 x 20 cm) hydroxyapatite column (Clarkson Chemical Co., Wilkes-Barre, Pa.). Run a 1 L gradient of 10-400 mM K-PO_4, pH 7.0.

12. Pool fractions with highest activity (the endonuclease elutes about 0.20-0.25 M K-PO_4) and dialyze against EB.

13. Concentrate by adsorbing to small DE-52 column (in Pasteur pipette) equilibrated with EB plus 10-20% glycerol. Elute with 0.25 M NaCl EB + 10-20% glycerol. Store as eluted at 4°C.

    Note: NaCl inhibits the endonuclease activity at concentrations above 0.1M NaCl.

Final prep should have about 25 mg protein, depending on column cuts, and enough endonuclease to cleave about 5 mg λ DNA.

ASSAYS FOR RI AND RII ENDONUCLEASES

1. Centrifugation assay. A reaction volume of 100 µl containing 100 mMolar TRIS, pH 7.5; 5 m Molar $MgCl_2$; about 1 µg of unmodified H^3 λ DNA and 0.1 to 1.0 µg of P^{32} modified DNA; with 1-50 µl of enzyme fraction.

   Incubate 15 min at 37°C and add 10 µl 10% SDS. Centrifuge 2.25 hr at 55,000 RPM in SW 56 rotor, linear 5-20% sucrose gradients in 0.1 m$KHPO_4$, pH 7.0, 0.1 M NaCl. Fractionate (15-17 fraction) and determine amount of H^3 sedimenting slower than P^{32}.

2. Use electron microscope to follow conversion of circular molecule of SV40 DNA to linear molecule. One double strand cleavage per molecule for RI endonuclease.

(1)

Betlach - Boyer Procedure for Preparing Closed-Circular DNA.

1) Cells are grown overnight, ~16 hrs, in brain-heart-infusion broth, 10 μg tetracycline·HCl/ml, 37 C.
2) Cells harvested by centrifugation, washed with ~~the~~ 100 ml 50 mM TRIS-HCl, pH. 8.0.
3) Cell pellet resuspended in a final volume of 30 ml with 25% sucrose, 50 mM Tris·pH 8.0
4). About 5 mg lysozyme added; kept on ice 15 min.
5) Add 1.5 ml 0.25 M EDTA, pH 8.0; on ice for 15 min.
6) Add 1.6 ml 20% SDS. Expel the SDS through the bottom of the cell solution to facilitate dispersal of the SDS. Gently mix the ~~lysing s~~ the solution which becomes very ~~viscous~~ viscous quite rapidly.
7) Add 8.4 ml of 5 M NaCl. MIX WELL & store on ice for at least one hour or overnight.
8) Centrifuge 35,000 RPM in 35 Fixed angle rotor, 4°C, one hour.
9). Decant the supernatant, taking care ~~not~~ to not include the very viscous secondary pellet.
10) Add an equal volume of H_2O; phenol extract and centrifuge at low speed ~~to get a~~ long enough to get a good aqueous phase. We find 5000 RPM for 1 hour at 4°C (Sorvall SS34 rotor) sufficient.
11) Remove aqueous phase & add 1/10 volume of 0.3 M NaAcetate and 2 volume of cold (-20C) 95% ethanol.

12) ~~to~~ Keep it -20°C ~~for~~ 30-60 ~~min~~ minutes (or longer if you need to interrupt the procedure)

13.) Centrifuge Sorvall ~~GS~~ A, 10,000 RPM, -10C, 15 min.

14) Resuspend in 10 ml of 0.1M Na_2HPO_4-NaH_2PO_4 pH 6.8, 0.6 M NaCl.

15) Adjust pH to 12.3 (pH 10 buffer standardized) 2N NaOH & stir for 3 minutes. Neutralize with 2N HCl.

16) If cloudy, low speed centrifugation ~~with~~ ~~on~~ is necessary. Keep cold to reduce renaturation of single strand DNA.

17) Filter supernatant through B6 Selectron (Schleicher & Schuell) nitro-cellulose filters. ~~These are~~ the filters are dropped into dilute ethanol (100% ethanol + 2 volumes of H_2O), soaked for 15 minutes, & then washed in distilled H_2O. They are then placed in the phosphate-NaCl solution. We ~~put~~ stack three filters in a Millipore filter without the chimney. The supernatant ~~mixture~~ is added to the entire surface & under H_2O ~~aspirator~~ aspirator pressure collected in a glass test tube dropped in ~~the~~ an aspiration flask. When the flow rate slows because of ~~clogged pores~~ clogged pores, turn the top filter over ~~&~~ until it drains & replace it with a fresh filter ~~&~~ We usually replace the ~~other two~~ bottom two filters once or twice during this procedure.

18.) The filtrate is dialyzed against H_2O for 1 hour and flash evaporated to about 1-2 ml.

19.) An optional step at this stage is the addition of 10 μg of boiled RNase ē 15 min, room temp. We find this helps the resolution of the subsequent column step.

20.) The concentrated solution is applied to an A50 agarose (Bio Rad; 100-200 mesh) 2 x 20 cm column equilibrated in ~~the phosphate buffer - NaCl solution~~ 25 mM TRIS-HCl, pH 7.5, 0.6M NaCl. The DNA elutes in the exclusion volume & identified by OD or isotope counting.

21.) The fractions of interest are pooled & ethanol precipitated.

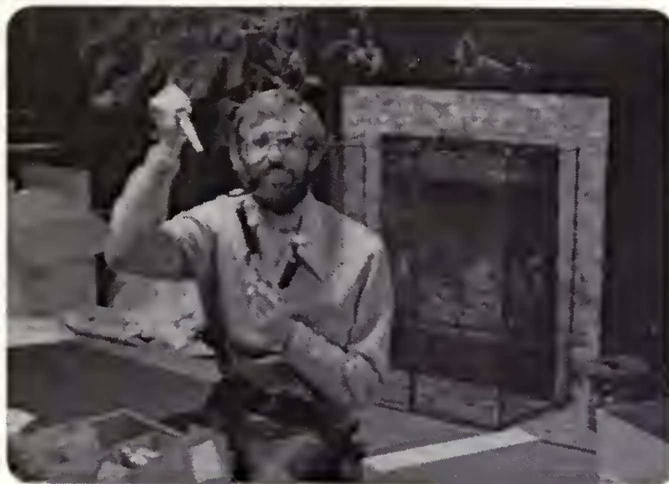
Yields for small plasmids (5-20 x 10^6 daltons) vary from 50-100 μg.

Three shots of Herb Boyer in his Microbiology Dept lab. One shot showing him in his office sitting at his desk. A second shot showing him in front of the fume hood, which has his running clothes hanging in it. A third shot of him flanked on the left by Steve Yanofsky (graduate student) and on the right by Keith Backman (postdoc).

The top right photo (dated 1977) shows Pat Greene (left) who was pregnant at the time and wearing a T-shirt labeled "Recombinant DNA" with an arrow pointing to her abdomen and me (right) in the Grateful Dead T-shirt. We had just competed in a Biochemistry Dept. running race in which the Boyer lab had just won the "beer relay race" event in which five of us had competed against other labs in a relay race characterized by drinking beer while you ran. The bottom right photo is of me in my flat at a lab party I had organized, which was a going away party for Keith Backman.

The three photos on the left of the page are from Keith Backman's going away party. In the photo at top we had given Keith a going away present which was in a small box labeled "recombinant DNA kit." Inside was a condom with a hole in it which Keith is displaying on his little finger. Others in the photo are Herb, Eli Elahi (Iranian postdoc), and Dave Russel (research associate) and his girlfriend. The middle photo shows (half of) Tom Kornberg (Biochemistry Dept. professor) and Jon Lawrie (postdoc). The bottom photo shows Keith Backman, John Rosenberg (professor and x-ray crystallographer collaborator on EcoR1 project), a postdoc from Tom Kornberg's lab, and Pat Greene (lower right). The photo on the right is of Esther Lederberg and Werner Goebel (professor from Univ. of Wurzberg in Germany who did a sabbatical in the Boyer lab in the late 70s).

The top two photos are from Keith Backman's going away party and show Herb drinking a beer next to Keith; and Herb standing next to Pat Greene. The bottom photo was taken at a lab party at an Italian restaurant in North Beach and shows Wes Brown and Herb.



The top photo is of Francisco (Paco) Bolivar who had been a postdoc in the Boyer lab and was back visiting. (At his going away party we gave him a hula hoop with the sequence of pBR322 DNA on it.) He was staying at my flat and had bought some lab equipment (pipetman, etc.) when visiting and was boxing it all up for his return to Mexico. Middle photo is of a lab party at Pat Greene's house. In the photo (left to right) are Linda Robinson (postdoc), Herb, me, Paco Bolivar, my son John, and a visiting scientist from Iceland (I can't remember his name). The bottom photo is of a lab Thanksgiving day party (~1977) at my flat. On the left side of the table are Will Spiegelman (Pat Greene's husband) and Keith Backman. On the right side of the table are Dan Vapnik (visiting scientist in the Boyer lab at the time; later of Amgen fame), Jon Lawrie (postdoc), and Kirby Alton (worked for Dan V.).

Top two photos taken at lab party at Pat Greene's house. Top photo back row shows wife of Icelandic postdoc, Herb, Franny DeNoto (Howard Goodman's research associate), Keith Backman, daughter of Icelandic postdoc, and Icelandic postdoc. Front row shows Linda Robinson (postdoc), Paco Bolivar, and my son John. Center photo shows Herb and Pat Greene. Bottom photo shows Herb in his new biochemistry lab on the 15th floor.

Photos from new Boyer lab on the 15th floor. Top photo shows Herb, Pat Greene, and Keith Backman. Center photo shows unknown graduate student, David Russel, Axel Ulrich (Goodman lab postdoc), Herb Boyer, Icelandic postdoc and Keith Backman.

Bottom photo shows Axel Ulrich, Franny DeNoto, and Herb clustered around my lab desk in the lab, drinking and eating.

Top photo shows Boyer lab meeting on the 15th floor. Left to right: Jon Lawrie, Kirby Alton, Jordan Konisky (visiting scientist), Pat Greene, me (with beer can in front of face), Keith Backman (in front of me), Jan Maat (postdoc from Netherlands), Eli Elahi, and I don't know who the guy is behind the pole. Middle photo is of Kirby Alton and Dan Vapnik working in the 15th floor lab. Bottom photo is of Madhu Gupta (Pat Greene's research associate), Pat Greene, and me in front of my desk in the lab.

These were taken for the Smithsonian article and show (clockwise starting upper left) Herb looking at a bacterial plate; Ray Rodriguez collecting bands from a CsCl gradient for a plasmid prep; me looking at a bacterial plate; me tracing plasmid DNA spreads in the Electron Microscopy room; and Herb looking at bacterial plates.

Mary Bottalch

R. Helling
April 1973

AGAROSE GEL ELECTROPHORESIS OF LINEAR DUPLEX OF DNA

Double-stranded linear DNA of molecular weight 1×10^5 and higher is routinely separated on 0.7% agarose gels. There is a linear relationship between log molecular weight and mobility up to $5 - 6 \times 10^6$, and separations and molecular weight estimates of larger DNA can be made. Molecular weights are estimated from internal DNA standards such as endonuclease RI fragments of ϕ80 and λ. The error in molecular weight estimate is less than 5% in the linear range. Where the DNA of interest is below $1 - 2 \times 10^6$ MW, higher concentration agarose should be used. Relative mobilities are unaffected by temperature over the range 3°C - 24° C (although absolute mobility increases with temperature). Thus separation appears to be based on molecular size and not on base composition.

TEA - NaCl Buffer

40 mm Tris acetate, pH 8.05

20 mm Na acetate

2 mm Na_2-EDTA

18 mm NaCl

Gels

15 x 0.6 cm gels are formed in disposable 5 ml pyrex pipets, cut so as to leave a constriction at the bottom (to retain the gel). (Seakem) – Agarose in buffer is melted in the autoclave. A small amount is pipetted around the outside of the tip of the tube (held in a rack) to seal it, and after hardening, additional agarose is

added to form a 15.5 cm column. The gels are allowed to harden for at least 40 minutes. Subsequently, the upper end of each is extruded (using a water-filled bulb to avoid air bubbles) and sliced evenly to form a 15 cm gel.

Loading Sample and Running

The sample is heated 5 minutes at 65° C to separate loosely associated DNA molecules (e.g., "sticky ends"), and quenched on ice. Bromphenol blue and sucrose are added (to 20% sucrose) to give a final sample volume of 20 μl (optimally) to 100 μl.

The sample is run into the gel 5 minutes at 100 v, and then at 22.5 v (1.5 v/cm gel). After about 20 hours at room temperature the dye marker should be at the gel tip. The dye mobility is equivalent to that of DNA of 1 - 3 x 10^5 MW.

Staining

The gels are extruded into 5 μg/ml ethidium bromide. After a half hour the stained bands may be visualized over long wavelength ultraviolet light ("black light"). (Protective glasses should be worn.) A band containing 50 - 100 ng is prominent; as little as 10 ng can be seen.

The gels are immediately photographed beside a ruler, using a yellow (Kodak No. 9 Wratten gelatin filter) or orange filter, and measurements are taken from the prints (Polaroid 55 P/N or 52).

Mary Betlach

ELECTROPHORESIS OF DNA IN AGAROSE GELS

BUFFER: Tris-Borate

	10.8 g	Tris base
	.93 g	Na_2 EDTA
	5.5 g	Boric acid
Bring to	1.0	liter

Agarose. SeaKem (MCi Biomedical: Division of Marine Colloids, Inc.
P.O. Box 748
Rockland, Maine 04841)

The appropriate concentration of agarose is dictated by several factors. We usually use 0.7% to 1.2% agarose made up in the Tris Borate buffer. The agarose is melted by autoclaving for 5 min, refluxing or simply heated in a boiling water bath.

Agarose gels can be made in standard tubes or slab apparatuses. Tubes can be made from 5 ml disposable pyrex pipets or glass tubing. Cut the delivery end of the glass pipet to leave a tapered end which prevents the gel from slipping out of the tube. Stands are available for placing the tubes in an upright position with the tip of the tube in a small depression in the stand. Add molten agarose to the depression and allow to harden before filling the tube with agarose. After solidification of the gel, the top must be sliced evenly to provide a horizontal flat surface for the sample. This can be done by filling a small rubber bulb with H_2O or buffer, and use the bulb to force the gel part way out of the tube which can then be cut with a razor blade, etc., and retracted into the tube. Standard electrophoresis chambers for tube gels can be used.

Slab gels can be made with the Studier or EC apparatus. Standard power supplies with the proper connections are sufficient. The sample is diluted with a Brom-phenol-blue-glycerol-SDS solution (.07%, 33%, 7% SDS; 1 part glycerol solution to 5 parts sample). Sample volumes are determined by the area of the sample slot. Usually the sample volumes for the tube gels are not greater than 50 µl. For large preparative slab gels one can apply ml quantities.

Electrophoresis conditions are variable. For best resolution of DNA fragments on tube gels we use a constant voltage of 1.5 V/cm of gel, which is usually around 22-23 V. Running times for fragments of 0.5×10^6 - 20×10^6 amu are about 24 hr, room temperature. Preparative slab gels are usually run at 75V for 18-20 hr. Analytical gels for fast separation of fragments with large mobility differences is 1 - 1.5 hr.

Gels are stained by soaking in ethidium bromide (∿ 1 µg/ml in buffer or H_2O for about 5 - 10 min. The fluorescing DNA bands can be visualized on a long-wave or short wave transilluminator (San Gabriel UV Products). [Note: We routinely use plastic or rubber gloves to handle the stained gels since Dr. B. Ames informed us that ethidium bromide has the molecular features of many carcinogens.] Photographs and

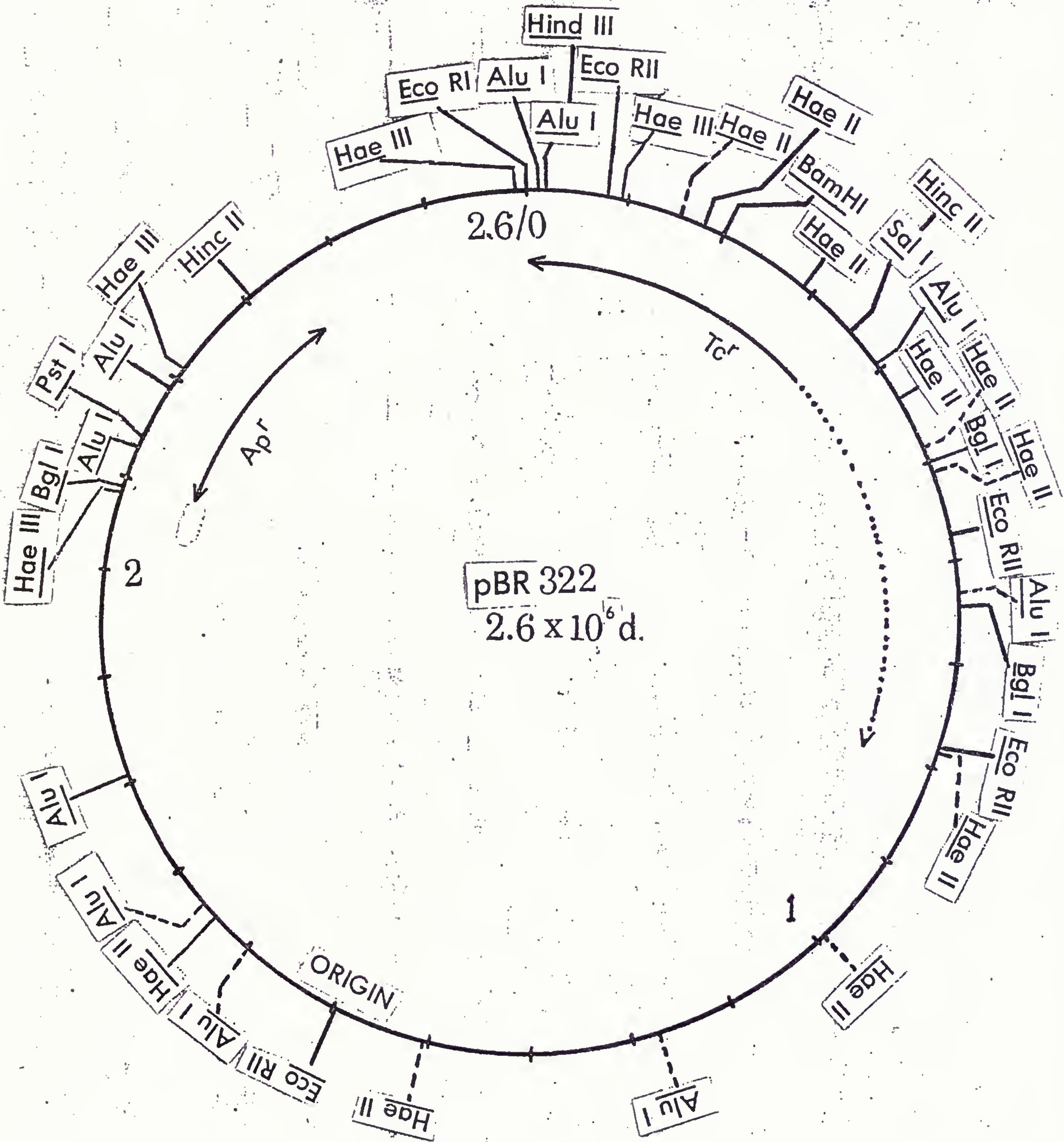
negatives can be made with a standard Polaroid Camera set-up for making lantern slides. Use NP 55 positive and negative film which are exposed in a #545 Land Film Holder. A double filter gives the best contrast. A J344 filter (San Gabriel UV Products) is placed closest to the UV source, and the second filter is a #9 Wratten yellow filter; with the diaphragm wide open, exposures of about 10-15 sec should be sufficient. Negatives are prepared as described by Polaroid. These can be used for reproductions and for measuring relative mobilities of gel fragments. The latter is done by enlarging the image and making a tracing of the projected image.

Gels can be stored in the cold (if wrapped in saran wrap) for 24-48 hr without too much diffusion of DNA fragments in 1 - 10 x 10^6 amu range.

REFERENCES

1. Sharp, P., Sugden, W. and Sambrook, J. (1973), Biochemistry 12, 3055-3063.

2. Cohen, S., Chang, A., Boyer, H.W. and Helling, R. (1973), Proc. Natl. Acad. Sci, USA 70, 3240-3244.



Hind III
Eco RII
Eco RI
Alu I
Alu I
Hae III
Hae III
Hae II
Hae II
BamHI
Hinc II
Hae II
Sal I
Alu I
Hae II
Hae II
Bgl I
Hae II
Eco RII
Alu I
Bgl I
Eco RII
Hae II
Hae II
Alu I
Hae II
Eco RII
ORIGIN
Alu I
Hae II
Alu I
Alu I
Hae III
Bgl I
Alu I
Pst I
Alu I
Hae III
Hinc II
2.6/0
1
2
Tc^r
Ap^r
pBR 322
2.6 x 10^6 d.

MIDNIGHT HUSTLING, INC.

HARVARD UNIVERSITY
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June 1, 1977

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It has come to my attention that you are in arrears for your BLMH subscription. Please remit $4,000,000.00 or one insulin producing clone immediately.

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Circulation Manager.

lj/

EXTRA - EXTRA SPECIAL EDITION MAY 31, 1977

BIOLABS MIDNIGHT HUSTLER

CITY OF CAMBRIDGE
MASSACHUSETTS

ALFRED E. VELLUCCI
MAYOR

May 16, 1977

Mr. Philip Handler
President, National Academy
of Science
2101 Constitution Avenue, N.W.
Washington, D.C. 20418

WALTER L. MILNE
MAY 18 1977
ROOM 5-208

Dear Mr. Handler:

As Mayor of the City of Cambridge, I would like to respectfully make a request of you.

In today's edition of the Boston Herald American, a Hearst Publication, there are two reports which concern me greatly. In Dover, MA, a "strange, orange-eyed creature" was sighted and in Hollis, New Hampshire, a man and his two sons were confronted by a "hairy, nine foot creature" (see attached news photo from the Herald-American).

I would respectfully ask that your prestigious institution investigate these findings. I would hope as well that you might check to see whether or not these "strange creatures," (should they in fact exist) are in any way connected to recombinant DNA experiments taking place in the New England area.

Thanking you in advance for your cooperation in this matter, I remain

Very truly yours,

Alfred E. Vellucci

Alfred E. Vellucci
Mayor

AEV:mt
Enclosure

Boston Herald-American photo

✪ BIOLABS MIDNIGHT HUSTLER ✪

READ ALL ABOUT IT – WHAT'S NEW, WHAT'S UP, AND WHAT'S HAPPENING!

MONEY AND FAME FROM CHICKENS

WG SAYS SALT TALKS PRODUCTIVE (★★★★)

★NOSTALGIA...

The way it was

SEX AND SEXISM IN THE XEROX ROOM

HUBBARD SPEAKS OUT FOR NEW YOUTH DIET

Dear DEbbiEE

KINKY KORNER

THE CASE OF THE POSTHUMOUS POST-DOC

CLASSIFRIED ADS

MONEY AND FAME FROM CHICKENS

On the road to Stockholm

(UPI) Cambridge, MA, April 1, 1977--The American Association of Pseudo-intellectuals today lauded the achievement of Dr. William A. Haseltine of the Sidney Farber Cancer Center, Boston, Massachusetts. After a search of many years Dr. Haseltime has found a way to get his name into the pages of the New York Times, and in so doing to cure cancer in chickens. The scope of this achievement has stunned the scientific community here. "I never thought he'd get farther than Science for the People," an unidentified source at the Center for the Study of Transient Fame said yesterday.

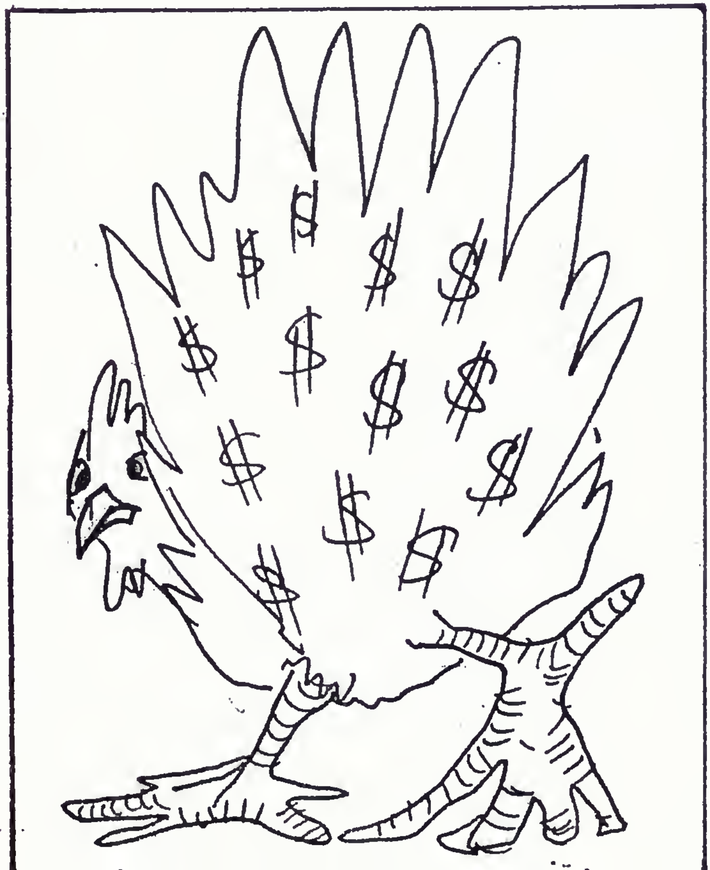
A source at the Sidney Farber Center described the ingenious cure at a news conference held yesterday at Harvard's Phillips Brooks House. It essentially involves feeding the affected chickens massive doses of a chemical called "Magic Spot" isolated by Dr. Haseltine. The chemical is obtained from the bodies of graduate students that have died from the the strain of collaboration with Dr. Haseltine, and is administered to the chickens daily for a period of two months. "If the chicken can survive that, it can survive anything" the source was quoted as saying when asked to explain the mechanism of the cure.

The response to the cure has been overwhelming. The Institute of American Geeks has awarded Dr. Haseltine its Frank Perdue Memorial Poultryatism Award. Dr. Walter Gilbert stated yesterday that the cure was "intuitively obvious"; Nobel Laureate Dr. George Wald when contacted at the International Symposium for Obfuscation in Science stated "I never understood a word he said." Response from the rest of the scientific world has been equally gratifying to Dr. Haseltine.

A CHICKEN IN EVERY TEST TUBE

FOWL PLAY BY MOLECULAR BIOLOGIST AND NCI CHICKEN VIRUS SUPPLIER IN MASSIVE GET RICH QUICK SCHEME

Chicago Mercantile Exchange...March 31, 1977...Iced broilers led the plunge today in the commodity pits, down the limit to 6¢ a lb. The decline, a surprise to many traders, was related by Mike Chirigos, analyst with Jack Gruber Associates of Bethesda, to the recent FDA decision allowing St. Petersburg processor William Beard access to the iced broiler market. Dr. Beard's chickens had been termed "bloodless" by the rest of the industry, but the FDA found Beard's suggestion to dump 8000 chicken carcasses a week on the market "entirely legal." In other trading, platinum futures closed slightly higher, due to increased demand by DNA sequencers.



WG SAYS SALT TALKS PRODUCTIVE (****)

Salt City, Utah. March 25, 1977
After the first full day of negotiations in the new round of SALT talks, Harvard delegate W. Gilbert said the discussions had been useful. Because heads of labs are attending the current SALT meeting, it was expected that only issues of paramount importance would be brought up, and this proved to be the case today. "The first order of business," Gilbert reported, "was sodium chloride, and there was general agreement that 0.0666 molar concentrations are not required by restriction enzymes." This was the salt breakthrough everyone had been waiting for, and which had eluded previous SALT negotiators. When questioned by a Hustler columnist as to why it had taken so long to reach an understanding on salinity of buffers, Gilbert's only reply was, "Your column needs more salt."

With regard to on-site DNA inspection, a salient proposal was set forth by K.G.B. Skryabin, one of the Moscow representatives to SALT. "It is a well known fact," he pointed out, "that the hydrazine-powered deoxynuclear cruise missle requires salt to suppress T-cells in its nose cone." Succint as it was, this statement of the Soviet position admittedly confused Gilbert and the Harvard observers, who adjourned to study the proposal with the view that it turned on detection of cytosine residues in table salt by their smell. An announcement of the Harvard response is expected later today after consultation with experts back at the salt mines in Cambridge.

HELPFUL HINTS FOR LABORATORY GARDENERS

Now that Spring is on its way and you can open your lab windows, you can't excuse the pitiful appearance of your lab plants by blaming the terrible fumes in the rooms. (After all, you're still alive aren't you?) Here are some helpful hints for making your lab plants look healthy and green:

1) Water them only when the leaves begin to droop.

2) Make your watering solution 1M KCl; this serves to make the osmotic pressure in the soil greater than that in the leaves of the plant thereby withdrawing water and causing the leaves to droop. You are then kept busy by constantly watering your plants.

3) Spray the leaves with a mister at least once a week. A 1/100 solution of Creosol Green and water gives a vibrant, green appearance to the foliage.

4) At odd intervals during the day and night flick your lab lights on and off rapidly. This will help your flowering plants blossom more profusely by giving them more than one photoperiod a day.

Lab regulation

ONLY I AM AUTHORIZED TO AUTHORIZE AUTHORIZATIONS. ANYONE WHO AUTHORIZES AN AUTHORIZATION MUST HAVE IT AUTHORIZED BY ME.

PAT

☆NOSTALGIA....

The way it was

Cambridge, Ma...March 31, 1967...The Cambridge social scene was highlighted by the party last weekend at Prof. Jim Watson's Apian Way apartment, given for Mario Capechi, the junior fellow who finally decided to write up his thesis. Some particularly intersting couples were noted, not the least of them involving Jim himself (currently one of Cambridge's most eligible bachelors) and Lorraine Larrison, a post-doc in Denhardt's group. Lorraine came over early for dinner, before the party got going, but our reporter was there to take in the scene. Dave Dressler arrived with Ursula Johnson, and Chris Weiss with a non-scientist. (Young Janice Pero came unescorted.) Once the music and dancing got underway, it was just like any typical Cambridge party. A group of party crashers, led by Mike Sinensky, a first-year graduate student in Biochemistry, tried to enter around midnight, but Jim rose to the occasion and threw them out. Sinensky, laboring under the false impression that this was an official party of the department, identified himself as Mike Sinensky, first-year graduate student. Jim was unmoved, and undoubtedly remembered the name.

SEX AND SEXISM IN THE XEROX ROOM

OR WHAT GOES ON WHILE WAITING FOR THE MACHINE TO WARM UP

Cambridge, Ma...Hysterical reports of "half-man, half-alligator" recombinant DNA monsters continue to filter out of the Biological Laboratories despite a tight curtain of secrecy imposed by the director of the labs, "Ironpants" Pollitt. Professor Ruth Hubbard called today for a full investigation, claiming that female staff members have been assaulted while using copying machines and in photographic darkrooms. Pollitt, however, reports that all cases of assault can be traced to normally libidinous male faculty members. Visiting lecturer Dr. Jeffery Miller termed the situation "seductive." Reached for comment while attending the World Health Organization seminar on cannibalism in Papua, New Guinea, Dr. Walter Gilbert called Hubbard "irresponsible." "It's a known fact," Gilbert was quoted as saying, "that the description of these so called monsters fits many of my graduate students and postdoctoral fellows."

BLMH PUZZLE

Unscramble the following words. Then use the circled letters to spell out a two-word phrase.

R (A) C E N G I (N) C O
L (E) D R I M A Y C A
L A (B) Y L
Y O (T) B A R O L A R
(S) I T E S (H)
H A N (W) M A T

Quotation of the week

"My i^{Q} is -35."

paper of the month:

A new method for promoting DNA

(DNA chemistry/dimethyl sulfate cleavage/hydrazine/piperidine)

ALLAN M. MAXAM AND WALTER GILBERT

Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138

Contributed by Walter Gilbert, December 9, 1976

HUBBARD SPEAKS OUT FOR NEW YOUTH DIET

March 28, 1977. The National Enquirer reported today that Dr. Ruth Hubbard of Harvard University is the first U.S. scientist to speak out in support of Dr. Frank's controversial youth diet. Dr. Frank's diet is based on the use of RNA, a molecule chemically related to DNA. The diet is considered controversial because tests leaked from Canada indicated that RNA induces tumor formation in rats. Dr. Hubbard, in an informal interview, gave her personal testimonial. "This diet is the fountain of youth. I've been on it for 15 years--I'm living proof!" She also stressed that rumors of recombinant RNA experiments were totally false. "No one at Fort Detrick ever died while on this diet," she stated.

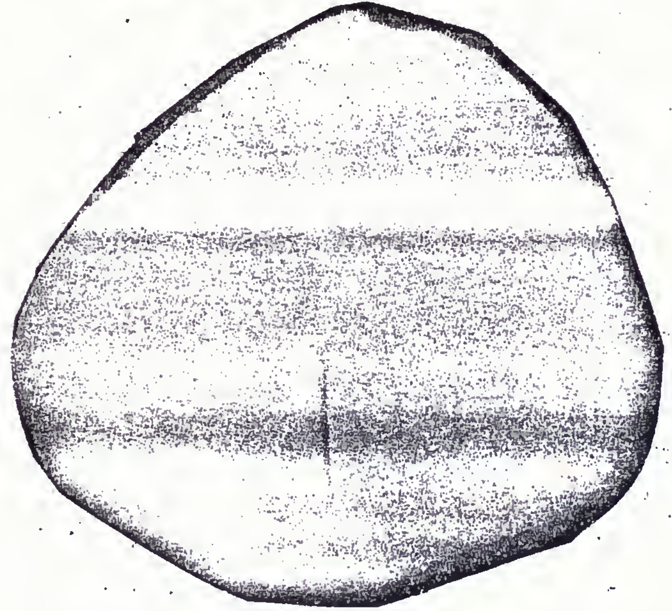
DR. HUBBARD'S
REG. U. S. PAT. OFF.
Vegetable
BRAND
GERMICIDE

This product has no connection whatsoever with American National Red Cross.

AN ANTISEPTIC
NON-POISONOUS GERMICIDE
CONTENTS 3 FLUID OUNCES
ACTIVE INGREDIENTS
85% Alcohol, 10% Mixture of Thymol, Oil Origanum, Oil Cajeput, Oil Lavender, Oil Rosemary and Oil Pine, 5% Inert.
ATOMIZER COMPLETE

J. Hubbard Co., Inc.
Nashua, N. H.
ESTABLISHED 1889

KINKY KORNER



The above questionable object was discovered in the abandoned laboratory of Dr. T. Maniatis. Conclusive identification has proven to be more difficult than expected, but the experts have offered some persuasive hypotheses:

W. Gilbert: Half-eaten nude mouse.

A. Efstratiadis: It's Jonathan King.

M. Ptashne: Where's the key to the Xerox room?

A. Maxam: Looks like a G, but it could be an A.

C. Williams: Why that's an Assonorium pharoanis

M. Pasek: Two-fold rotational symmetry.

A. Worcel: Nu body.

Have You Been Bited in Cell?

"GEL CRAP!"

Dear Debbie

Dear Debbie:
I'm in love with a geneticist from Geneva, although sometimes I think he's lacking. Talk about the cowboy kissing the horse, he sleeps with his strain collection under his pillow and he won't even kiss me because he says we can't use sterile technique. Is this a hopeless romance?

I⁻

Dear I⁻:
I wouldn't say it's hopeless, but it sounds as though your romance is in need of an active promoter. You might try telling him that some of the most interesting bugs around result from using non-sterile technique, and if that doesn't work, you could threaten to put saltpeter in his broth. As for his sleeping with his strain collection under his pillow, don't complain too much, my dear. There's one woman who wrote me that her husband sleeps with a lighted cigar in his mouth and that she counts smoke rings in order to go to sleep. But if worse comes to worst, better move upstream to a faster operator.

Dear Debbie:
My old lab buddy has left me, and a new one has moved in. My problem is that the new buddy is a neat freak (i.e., anal.) What can I do about it?

Sloppy but Happy

Dear Sloppy but Happy:
My anal-ysis of the situation is this: the core of the whole problem will be for you to learn to be happy with your own mess and not depend on others to create disarray for you. Since you didn't outline the sequence of events which caused your old lab buddy to leave, they may have been rather traumatic, and you may still feel denatured. Give yourself some renaturation time; your neat freak lab partner may test out okay. In the meantime, continue to be happy while Piling it higher and Deeper.

Dear Debbie:
I'm an investigator--a private investigator these days. Recently I have been trying to tail a plasmid, but every time I get close, it undergoes some strange transformation and I loose it. I've tailed it in Cacodylate and in Cobalt, I've even tried a p-32 homing device. Nothing seems to work! Well, if you think this sounds bad, you should try tailing a lac fragment with the "dT's." I know there's some kind of promotion operation upstream but it's a puzzle - the pieces of which haven't yet annealed. Any advice?

Turning Blue on an XG Plate

Dear Turning Blue on an XG Plate:
Get out fast!

Dear Debbie:
Why do we have glass pipes in our hallway?

Unsigned

Dear Unsigned:
The better to see it with, my dear.

And now I'm going to take a poll that I'm sure will interest everyone. The question is, "If you had it to do over again, would you go to graduate school?" Answer only on 3 x 5" notecards please. Simply state "Yes" or "No," and indicate your year in graduate school. If your answer is "No," please state what you wish you had done instead. The cards can be deposited in the "Dear Debbie" box outside Room 386, and the deadline is the day before the next edition of the Biolabs Midnight Hustler.

PRINT THIS Letter

or i'll

BLOW UP TExas

Walter Gilbert

THE CASE OF THE POSTHUMOUS POST-DOC

Another BLMH crime-stopper story in the continuing saga of WG-man!
by Agatha Crick-y

The man walked purposefully down the dimly lit hall. Dressed in a crumpled green suit, he had a firm, proprietary tread. He had a job to do. He wasn't sure what the job was yet, but he knew that by the following morning, a murderer would be caught, a burglary stopped, a spy discovered: the job would be done. Suddenly, he ducked into the darkroom and shut the door. Five minutes (with agitation), six minutes, seven minutes --and he emerged, dressed in sandals, jeans shorts, a bright orange shirt, a long, flowing purple cape and glasses removed. The Green Hornet? Wonder Woman? No, it's--WG-man! By day, a mild-mannered biochemist and molecular biologist, selflessly serving humanity seeking the cure for cancer (or the Nobel Prize, whichever comes first). By night, the GREATEST CRIME-SOLVER OF THEM ALL!!

Last week, we left our hero at the Moon Villa, celebrating with a pastry lunch the capture of the notorious Reznikoff Ripper and his gang. But today was another day. WG-man strode into his office, swept papers and journals off his desk, climbed up and struggled to open the window. Just as it opened, he heard a strange and ominous sound. "That sounds strange and ominous," he noted sagely and clambered off his desk, unanswered letters and fluorescent chalks flying as he fell. Picking himself up, he noticed when he squinted that a pair of shoes were sticking out of the doorway to room 386. Intrigued, he went toward them, musing, "Hm, it seems my post-doc has left his shoes in the lab." As he approached the doorway, his body tensed. The post-doc's shoes were there, alright, but with the post-doc still wearing them! The post-doc was flat on his back, possibly dead, but WG-man couldn't tell--for there was a 300-lb. crystal of repressor-operator complex on the chest of the junior scientist. "Heavens to Betsy!" exclaimed WG-man, though he quickly corrected himself, remembering that she had left. "By Benno," he gasped and this time grabbed the microscope to see the size and shape of the wonderous crystal. "Holy coli!" he shouted, "That's a 300-lb. crystal of repressor-operator complex!" Then he remembered the post-doc crushed beneath it. Before the next and immortal words--"What's up?"--could even be uttered, WG-man found his cape was being thrown over his head and tied securely around his waist. HE WAS CAUGHT!!

Who had been so clever as to trap WG-man at his weakest--when his back was turned? What nefarious mind had created the much-wanted crystal to bewitch his faculties and lower his defenses? Who didn't know that he was blind without his glasses anyway? And--what did this latest mishap have to do with the brutal murder of his colleague and young friend? What next in this sinister plot against our hero, WG-man?

Be sure to read the next issue of the Bio Labs Midnight Hustler!

WANTED

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(tea room)

"There's no place on earth like Greece."

—Dr. F. C. Kafatos

CLASSIFRIED ADS

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The LATEST in nonsexist automatic pipetting--

the PIPETTE INDIVIDUAL

Endorsed by Emily Friedan's mother and sold to you by a salesperson formerly an editor of Ms. Why let other labs be groovier than you? Order today and receive free your own autographed copy of Fear of Flying and a flammable brassiere (suitable for burning).

Harvard Biological Laboratories
16 Divinity Ave
Loony Bin
March 28, 1977

Classifieds Editor
Biolabs Midnight Hustler
388 Biolabs
Cambridge, Mass
USA

Dear Sir or Madam (whichever you prefer, no skin off my nose),

I would be very grateful if you would publish the following classified ad. Needless to say, I would appreciate your discresion (sp?) in this matter.

WANTED: WM, 5G, companionable, seeks lab partner. Interests include gerbil propagation, biochemistry. Object, co-existence. Reply MH box PE40.

Regards,

PJF

✪ BIOLABS MIDNIGHT HUSTLER ✪

U Know who

Courtesy Biolabs Tribune

LOOSE TALK CAN COST EXPERIMENTS

DEFENDING THE LAB REQUIRES BUTTONING ONE'S LIP AT MEETINGS

DNA Butt-Scuttle

Well, well recombo-readers, guess who's name is appearing in that imfamous gene maker magazine, Gene Gene Gene-Jelly Been again this week? That's right, Berb Hoyer and Rill Butter once again. This time the lucky number is three -- are you ready -- Full Length Reverse Transcript of Bovine HnRNA, Selection and Amplification of Bovine Secretory Control Genes, and Bacterial Expression of Bovus Lactus Locus! Hoyer said that the full significance of this work would be realized only after Micro-Milkers had been developed-- "They're cute, but they're small," he said. Another researcher questioned about this retorted, "That's Udder Bullshit." We won't mention any names but his initials are G.W.-- not necessarily in that order.

Well, we don't know much about that, G.W., but it does seem to this journalist that Hoyer is trying to clone the 102nd element in his hat -- the kind made of Au and indiginous to Stockholm.

Meanwhile, over in France -- P. Tiollais announced the completion of the whole human clone precipitation test. He's now trying to work out a method for resuspending the precipitatedd anti-Body. His colaborator, Kourilsky, was unable to comment from under the cover of his agar plate. I hope he's good at anaerobic growth.

Ta-ta for now Chimeric fans.

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTE OF HEALTH

TO : Director, NIH
Through: ES/NIH
Deputy Director for Science, NIH B. J.

DATE: July 6, 1977

FROM : Director
Office of Recombinant DNA Activities, NIGMS

SUBJECT: EK2 Host-Vector Systems Based on χ1776 and Plasmids pBR313 and pBR322

Dr. Herbert Boyer of the University of California, San Francisco, and Dr. Stanley Falkow of the University of Washington submitted data on December 20, 1976, on proposed EK2 host-vector systems based on *E. coli* K-12 strain χ1776 and plasmids pMB9, pBR313 and pBR322 (Attachment I). The data were reviewed by a subcommittee of the Recombinant DNA Molecule Program Advisory Committee on January 14, and by the full Committee on January 15. The Committee recommended that χ1776 (pMB9) should be certified as an EK2 host-vector system, and you certified χ1776 (pMB9) as an EK2 system on April 18.

The systems χ1776 (pBR313) and χ1776 (pBR322) tentatively were recommended for certification pending the receipt of further data from Drs. Boyer and Falkow concerning mobilization and transmission of these two plasmids. The Working Group for EK2 Host-Plasmid Systems, which met on March 21, prepared a report (Attachment II) which concluded that certain additional test data for pBR313 and pBR322 would be needed in order to satisfy the revised requirements for submission of data on proposed EK2 systems (Attachment III).

Dr. Falkow supplied the required test data in a letter dated May 31 (Attachment IV).

The Recombinant DNA Molecule Program Advisory Committee reviewed the Working Group's report on June 23. The Committee voted 9 to 0 to approve the report and the "Instructions to Investigators Concerning Data to be Submitted on Host-Plasmid Systems Proposed for EK2 Certification." Dr. Curtiss abstained from the vote.

Dr. Adelberg summarized the data submitted by Dr. Falkow (Attachment V). He pointed out that all the required tests are met by these systems. The Committee voted 9 to 0 to recommend that χ1776 (pBR313) and χ1776 (pBR322) be certified as EK2 host-vector systems. Dr. Curtiss, whose laboratory was involved in the construction of χ1776, abstained from the vote.

Recommendation: I recommend that you certify χ1776 (pBR313) and χ1776 (pBR322) as EK2 host-vector systems.

William J. Gartland, Jr., Ph.D.

Attachments

Concurrence:

Donald S. Fredrickson, M.D. Dated July 7, 1977

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Sally Smith Hughes

Graduated from the University of California, Berkeley, in 1963 with an A.B. degree in zoology, and from the University of California, San Francisco, in 1966 with an M.A. degree in anatomy. She received a Ph.D. degree in the history of science and medicine from the Royal Postgraduate Medical School, University of London, in 1972.

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Presently research historian and principal editor on medical and scientific topics for the Regional Oral History Office, University of California, Berkeley. Author of *The Virus: A History of the Concept*, Sally Smith Hughes is currently interviewing and writing in the fields of AIDS and molecular biology/biotechnology.

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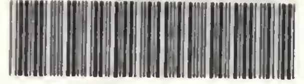
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